Chapter 3

Redox Chemistry of 8-Azaadenine: A Pulse Radiolysis Study

Abstract

The redox chemistry of 8-azaadenine has been investigated using pulse radiolysis technique. The oxidation reactions were studied using hydroxyl radical ("OH), oxide radical anion (O^-) and sulfate radical anion (SO4^-), and the reduction reactions were studied using hydrated electron (e^-aq) and hydrogen radical (H^+). In the reaction of "OH, a bimolecular rate constant of $3.8 \times 10^{10}$ dm$^3$ mol$^{-1}$ s$^{-1}$ was determined at pH 6.0. The transient spectrum obtained for the reaction of "OH at pH 6 has an absorption maximum around 340 nm and is assigned to the formation of 8AA-4OH^+. The charge population density was calculated theoretically and it showed that the fourth carbon atom (C(4)) is the most probable site for the attack of "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 (using Gaussian 98) where the absorption maximum of this radical is calculated as 338 nm. The yield of 8AA-4OH^+ 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^+, which is an N-centered radical of the type 8AA-N(9)^+. In the reaction of O^- (pH = 14), the transient intermediate is also assigned to 8AA-N(9)^+. In the reaction of SO4^- at pH 6, the transient spectra having $\lambda_{max}$ at 320 nm is attributed to the formation of a neutral radical of 8AA (8AA-N(9)^+), which is formed by the deprotonation of the initially formed radical cation. But at pH 10.2, the spectra is found to be similar to the one observed in the reaction of O^- and hence it is attributed to the formation of the nitrogen centered radical 8AA-N(9)^+. In the reaction of e^-aq, (k = $1.8 \times 10^{10}$ dm$^3$ mol$^{-1}$ s$^{-1}$ at pH 6) the transient absorption spectrum with $\lambda_{max}$ at 330 nm is assigned to the protonated electron adduct of 8AA (8AA(NH^+)). The reducing nature of this intermediate is confirmed by the formation of methyl viologen radical cation (MV^+*) from its reaction with MV^+*. The transient intermediate in the case of the reaction of H^+ is proposed as 8AA-C2(H)N(3)^+ at pH 1.
3.1 Introduction

8-Azaadenine is one among the aza derivatives of purines, which are known to have cytotoxic, antimicrobial, and mutagenic properties. An earlier study reported that the water derived free radicals such as hydroxyl radicals (·OH) and hydrated electrons (e\textsubscript{aq}) react with aza analogues of pyrimidine at diffusion controlled rate \(10^9 \text{ - } 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}\) 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. For example; the potential sites of attack in the case of the reaction of ·OH with adenine are identified as C4, C5 and C8 positions. ·OH adds to C(4) (35% probability), to C(5) (19%) and to C(8) (30%) in the case of N\textsuperscript{6}, N\textsuperscript{6}-dimethyladenosine. The resulting radicals are represented as A4-OH, A5-OH and A8-OH, and all of these undergo unimolecular transformation reactions. The A8-OH undergoes a ring opening reaction (imidazole ring), which yields 5-formamido-6-aminopyrimidine type product (FAPy). However, in the case of caffeine only C(4) and C(8) OH-adducts are formed with a ratio of 1:2. While the formation of A8-OH 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\textsuperscript{−}), sulfate radical anion (SO\textsubscript{4}\textsuperscript{−}), hydrated electron (e\textsubscript{aq}\textsuperscript{−}) and hydrogen atom (H\textsuperscript{•}), 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.

3.2 Theoretical Calculations

The charge population density calculated for 8AA is shown in figure 3.1. 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. As C(4), C(5) and C(8) are the most potential sites of 'OH attack, only the charge population density of these three sites are shown in the figure. As the highest population density is at the C(4) position, it is likely the most probable site for the electrophilic attack of 'OH. The absorption maximum of the various radicals have also been calculated theoretically and are shown in table 3.1.
Figure 3.1: NBO Charge population (in au) of 8 Azaadenine (This demonstrates the C(4) (0.35 au) is the most probable site of electrophillic attack)

Table 3.1. Experimentally observed and theoretically calculated absorption maximum ($\lambda_{\text{max}}$) of various radicals derived from $^1$OH reaction

<table>
<thead>
<tr>
<th></th>
<th>$\lambda_{\text{max}}$ (Experimental)</th>
<th>$\lambda_{\text{max}}$ (Theoretical)</th>
</tr>
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<tbody>
<tr>
<td>8AA-4-OH$^*$</td>
<td>350 nm</td>
<td>337.77 nm $f=0.0088$</td>
</tr>
<tr>
<td>8AA-5-OH$^*$</td>
<td>-</td>
<td>318.96 nm $f=0.0192$</td>
</tr>
<tr>
<td>8AA-N(9)$^*$</td>
<td>300, 350 and 640 nm</td>
<td>325.53 nm $f=0.0001$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>335.60 nm $f=0.0863$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>628.41 nm $f=0.0020$</td>
</tr>
<tr>
<td>8AA-N(9)$^{**}$</td>
<td>-</td>
<td>332.72 nm $f=0.0360$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>304.90 nm $f=0.1144$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>532.68 nm $f=0.0374$</td>
</tr>
<tr>
<td>8AA-N(6)$^*$</td>
<td></td>
<td>296.17 nm $f=0.0767$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>487.48 nm $f=0.0272$</td>
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<tr>
<td></td>
<td></td>
<td>444.25 nm $f=0.0221$</td>
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$f=$ oscillator strength
3.3 Reactions of Hydroxyl Radicals (·OH)

The transient absorption spectrum recorded at 5 μs 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

![Graph showing k_{obs} versus concentration](image)

**Figure 3.2:** $k_{obs}$ versus concentration plot obtained from the reaction of ·OH with 8-azaadenine at 340 nm at pH 6. Inset: Absorption trace at 340 nm obtained when the concentration of 8AA was $4 \times 10^{-4}$ mol dm$^{-3}$.

of the transient ($k_{obs}$) was found to be linearly dependent on the concentration of 8AA. The build up of absorption at different concentrations of 8AA at 340 nm and the linear plot of $k_{obs}$ versus concentration is shown in figure 3.2. From this plot, 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$^{-1}$)\textsuperscript{13} and is well comparable to purine ($3 \times 10^8$ dm$^3$ mol$^{-1}$ s$^{-1}$).\textsuperscript{13,14} The lower rate constant compared to the adenine is understandable due to the presence of a nitrogen at the 8th position instead of carbon and the 'OH is electrophilic in nature.

The absorption spectrum obtained at pH 6 at 40 μs after the pulse has a well-defined maximum around 350 nm [Fig. 3. 3]. 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.\textsuperscript{7-9}
Figure 3.3: Reaction of \(^\cdot\)OH with 8AA at pH 6 & 10.2: Transient absorption spectra recorded in N\(_2\)O saturated aqueous solutions of 8azaadenine (8AA) (1 \(\times\) 10\(^{-3}\) mol dm\(^{-3}\)) at 40 \(\mu\)s after the pulse at pH 6(\(\triangle\)) and pH 10.2 (\(\bullet\)). Inset: (a) Formation trace at 350 nm at pH 6 (b) TMPD\(^{\bullet\bullet}\) build up at 565 nm from the reaction of the intermediate with TMPD at pH 6.

The spectral features at higher pH (pH 10.2) were very different compared to that at pH 6. The transient spectrum recorded at 40 \(\mu\)s after the pulse showed two distinct absorption maxima at 300 and 350 nm and a weak broad maximum centered around 640 nm [Fig. 3.3]. 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 "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. 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 of the radical cation of TMPD (TMPD'), which is typical of similar electron transfer from the oxidizing intermediate to TMPD', has been observed at 565 nm at pH 6 (Fig.3.3 inset). The yield of TMPD' was also calculated in terms of G(TMPD') and was found to be $4.5 \times 10^{-7}$ mol J$^{-1}$. The total yield of "OH (G("OH)) is calculated as $5.3 \times 10^{-7}$ mol J$^{-1}$ which is based on the concentration of 8AA and its second order rate constant according to the equation 3.1.15

$$G(S) = 5.2 + 3.0 \frac{(k_s[S]/\lambda)^{1/2}}{1 + (k_s[S]/\lambda)^{1/2}}$$

where $k_s[S]$ is the pseudo-first-order rate constant for reaction of a solute with OH and $\lambda$ the track recombination frequency, which can be taken as $4.7 \times 10^3$ s$^{-1}$. This expression describes the yield of
a secondary intermediate that should be initially produced at a particular solute concentration. \( G(S) \) is the scavenging function related to the \( \cdot \text{OH} \) lifetime.

Considering this \( G \) value, the observed \( G(\text{TMPD}^+) \) constitutes about 85% of the total reaction. A similarly high \( G(\text{TMPD}^+) \) of \( 5.0 \times 10^{-7} \text{ mol J}^{-1} \) was obtained at pH 10.2 as well, which constitutes about 94% of the total \( \cdot \text{OH} \) reaction.

One of the general phenomena observed with adenine, adenosine and their substituted derivatives is the 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 figure 3.3, therefore, represents a relatively stable species in the time scale of pulse radiolysis experiment. 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)-OH-C(5)-yl radical (8AA-4OH\(^+\)). The assignment of this radical structure can be supported from its reaction with TMPD where the clear electron transfer from TMPD (Fig.3.3) demonstrates its oxidizing nature. This oxidizing property of the radical can well be explained due to the presence of unpaired spin
density on N1, N3 or N8 in all the other possible mesomeric structures (Scheme 3.1). The existence of 8AA-4OH' is further supported by theoretical calculations where the absorption maximum of this radical is calculated as 338 nm (oscillator strength = 0.0088). This value matches well with the observed absorption maximum at 340 nm [see Fig.3.3]. 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 μs) and that the 8AA-4OH' is stable compared to its adenine counterpart. On the other hand, the possibility of the formation of 8AA-5OH' cannot be ruled out. However 8AA-5OH' can not be an oxidant unlike 8AA-4OH'. The yield of TMPD'' at pH 6 (G(TMPD'') = 4.5 x 10^{-7} mol J^{-1}) is about 15% less than the quantitative yield of 'OH. It is therefore, reasonable to assume that this reduction in the quantitative yield of TMPD'' resulted from the formation of 8AA-5OH'. However, it is unlikely that this can contribute significantly to the absorption spectrum due to its low yield. Moreover, the theoretical calculations show that 8AA-5OH' has an absorption maximum at 318 nm (oscillator strength = 0.0192). However, no such indication is obtained from the observed spectrum [see Fig. 3.3]. Assuming similar optical properties for both, 8AA-4OH' and 8AA-5OH', the absorption
coefficient of $8\text{AA-4OH}^+$ is approximately calculated as $1640 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ at 340 nm.

The expected dehydration from $8\text{AA-4OH}^+$ 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$^+$ ($G(\text{TMPD}^+) = 5 \times 10^{-7} \text{ mol J}^{-1}$) demonstrates the oxidizing property of the intermediate. The spectral behavior at pH 10.2 is very different compared to that at pH 6 [see Fig. 3.3]. The absorption spectrum with maxima at 300 and 350 nm is attributed to the dehydrated $8\text{AA-4OH}^+$, which is an N-centered radical and can be represented as $8\text{AA-N(9)}^+$ (Scheme 1). This result is, thus, very different from the reaction of $^1\text{OH}$ with adenine and its derivatives at higher pH where an $\text{OH}^-$ induced inhibition of $\text{OH}^-$ elimination of $\text{A-4OH}^+$ leading to a deprotonated radical $\text{A-4O}^-$ is reported. Because of this reason the yield of TMPD$^+$ is seen highly reduced at higher pH. The existence of $8\text{AA-N(9)}^+$ in the present case is further confirmed by the formation of a transient species from the reaction $O^+$ with $8\text{AA}$ which has very similar spectral characteristics (see section 3.4). The reaction of $O^+$ with nucleobases proceeds via an electron transfer reaction with the deprotonated $8\text{AA}$ at pH=14 ($8\text{AA}$ has a $pK_a$ at 11.5) leading to a
N(9)-centered radical. The OH\textsuperscript{-} induced dehydration reaction from 8AA-5OH\textsuperscript{+}, though formed low in yield, is equally likely.

Scheme 3.1

The so formed 8AA-N(6)+ (scheme 1) can be a very good oxidant similar to 8AA-N(9)+, which explains the high yield of TMPD\textsuperscript{2+} at higher pH. As shown in scheme 1, the dehydration reaction can lead to two possible structures such as 8AA-N(6)+ and 8AA-N(9)+. 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' is expected to be less than 15% (see previous paragraph) it can be concluded that the spectral contribution is largely from 8AA-N(9)

3.4 Reaction of Oxide Radical Anion (O')

The reactions of O', which is a conjugate base of 'OH, will be quite different from that initiated by 'OH. It is an oxidant (E°[O', H'/OH'] = 1.77 V). Its reactions are normally carried out at a pH greater than 12 as the pK, value of 'OH + H' is at 11.2. Therefore, the reaction of O' with 8AA is carried out at pH ≈14. A second order rate constant of 4.2 \times 10^8 \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1} is determined from the pseudo first-order absorption buildup of the intermediate with respect to the concentration of 8AA at 350 nm from the k_\text{obs} \text{ versus concentration plot (Fig. 3.4). The transient absorption spectrum at 40 \mu s after the pulse has shown similar features like the spectrum obtained from the reaction of 'OH at pH 10.2, with absorption maxima at 300 and 350 nm with a broad absorption centered around 640 nm (Fig. 3.5). 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 OH-adduct undergoes a first-order type decay and form a hydrated adduct. The electron transfer reactions from the intermediates to
TMPD was also carried out and a strong absorption build-up of TMPD$^{\ast\ast}$ (Fig. 3.5), very similar to the reaction of $^{\ast}$OH at pH 10.2, was obtained. At pH=14, 8AA is predominantly existing in its deprotonated form at nitrogen as it has a pKₐ at 11.5. The reaction of O$^-$ was reported to be very different from the reaction of $^{\ast}$OH as the former undergoes either electron transfer or a hydrogen abstraction reaction with adenine and other nucleobases.$^{16,19,20}$ It is therefore proposed that O$^-$ undergoes an electron transfer reaction at N9 and this results the formation of a nitrogen centered radical, 8AA-N(9)$^*$ (reaction 5). The deprotonated adenine (at N(9)) has similar reaction with O$^-$ at pH>13.$^{18}$ In the case of O$^-$ a similar TMPD$^{\ast\ast}$ build up is observed as in the reaction of $^{\ast}$OH. The formation of TMPD$^{\ast\ast}$ can be well explained based on the oxidative nature of 8AA-N(9)$^*$ 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$^*$ at pH 10.2 (see scheme 1). An absorption coefficient of 2670 dm$^3$ mol$^{-1}$ cm$^{-1}$ is calculated for the 8AA-N(9)$^*$ at 300 nm.
Figure 3.4: $k_{\text{obs}}$ versus concentration plot obtained from the reaction of $\text{O}^\cdot$ with 8-Azaadenine at 350 nm at pH \approx 14. Inset: A typical absorption build up trace at 300 nm.

Figure 3.5: Reaction of 8AA with $\text{O}^\cdot$: Transient absorption spectrum recorded in N$_2$O saturated aqueous solutions of 8azaadenine ($1 \times 10^{-3}$ mol dm$^{-3}$) at 40 \(\mu\)s after the pulse at pH \approx 14. Inset: Absorption build-up of TMPD$^+$ at 565 nm resulting from the reaction of the intermediate with TMPD.
3.5 Reaction of Sulfate Radical Anion (SO$_4^{\cdot-}$)

The SO$_4^{\cdot-}$ is a powerful oxidizing radical with an oxidation potential of 2.5-3.1 V/NHE. 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, which is a similar process like the direct effect of ionizing radiation. It has a well resolved absorption spectrum with two maxima at 350 and 560 nm (Fig.3.6).

In the present study, the reaction of SO$_4^{\cdot-}$ is carried out at near neutral as well as at basic pH. A second order rate constant of $2.1 \times 10^8$ 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 $^\cdot$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$^{-1}$) 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 due to the presence of additional nitrogen compared to adenine.

**Figure 3.6:** Absorption spectrum of SO₄²⁻ at neutral pH. (taken from Reference 22)

A comparatively weak transient absorption spectrum is obtained at pH 6 with a single maximum at 320 nm (Fig.3.7). 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.3.7). It is, however, noticeable that this spectrum matches well with the one obtained from the reaction of 'OH at pH 10.2 and of O" at pH ∼ 14 (Figs.3.3 and 3.5).
Figure 3.7: Reaction of $SO_4^{2-}$ with 8AA at neutral and higher pH: Transient absorption spectra recorded in $N_2$ saturated aqueous solutions of 8-azaadenine (8AA) ($1 \times 10^{-3}$ mol dm$^{-3}$) containing 2-methyl-2-propanol (0.2 mol dm$^{-3}$) and $S_2O_8^{2-}$ ($5 \times 10^{-2}$ mol dm$^{-3}$) at 40 μs after the pulse at pH 6 (Δ) and at pH 10.2 (●). Inset: Absorption trace at 300 nm at pH 10.2.

Being a very powerful oxidant, $SO_4^{2-}$ 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 $SO_4^{2-}$ is a radical cation, which possess a very short life time in aqueous solutions in the case of purine systems due to their high Bronsted acidity.$^5$ Therefore, they deprotonate very fast ($k > 10^7$ s$^{-1}$), resulting a neutral radical.$^7$ Furthermore, the $pK_a$ of the radical cation is reported as much
lower than 1.5. A general reaction (scheme 3.2) of SO₄⁻² with purines or pyrimidines is shown.

\[
P + SO₄^{2-} \rightarrow (P-SO₄^{-}) \rightarrow P^{2+} \rightarrow \text{OH}\cdot \rightarrow \text{H}_2\text{O} \rightarrow P-\text{OH}•
\]

**Scheme 3.2**

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 reaction with water or OH⁻ at basic pH to form the corresponding OH-adducts. If latter is the case, then the spectral features at pH 6 should match with the reaction of 'OH at the same pH. However, this is not the case as can be seen from Figs 3.7 and 3.3. Then the most likely mechanism is the deprotonation of the radical cation to form a neutral radical of the form 8AA-N^6• (scheme 3.3). The deprotonation of the radical cation of adenine derivatives at the N⁶-position 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 \(^{1}OH\) (at pH 10.2), O\(^+\) and SO\(_4\)\(^{2-}\) (at pH 10.2) are well comparable as can be seen from Figs.3.3, 3.5 and 3.7. It is therefore proposed that the initially formed radical cation reacts with OH\(^-\) leading to the formation of 8AA-4OH\(^+\) (and a minor fraction of 8AA-5OH\(^+\)) which on dehydration reaction to give rise to a nitrogen centered radical of the form 8AA-N(9)** as shown in scheme 3.3.

![Scheme 3.3](image-url)
3.6 Reactions of Hydrated Electrons (e_{aq}^-) and Hydrogen Atom (\textsuperscript{1}H)

In general, the purines have high intrinsic reaction with e_{aq}^- (k \approx 10^{10} \text{ dm}^3 \text{ mol}^{-1} \text{ s}^{-1}) due to the presence of electron deficient pyrimidic ring.\textsuperscript{13} 8AA has also shown similar reactivity. A second order rate constant of $1.8 \times 10^{10}$ 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 (Fig. 3.8).

**Figure 3.8:** The plot of $k_{obs}$ versus concentration obtained from the pulse radiolysis of N$_2$ saturated aqueous solution of 8 AA and 2-methyl-2-propanol (0.2 mol dm$^{-3}$) e$_{aq}^-$. Inset: (a) absorption trace of e$_{aq}^-$ in the absence of 8AA and (b) in the presence of 8AA ($1 \times 10^{-3}$ mol dm$^{-3}$) at pH 6 (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. 3.9).

**Figure 3.9:** Reaction of 8AA with eaq at pH 6 and 12. Transient absorption spectra recorded in N2 saturated aqueous solutions of 8 azaadenine (1 x 10^{-3} mol dm^{-3}) containing 2-methyl-2-propanol (0.2 mol dm^{-3}) at 3 μs after the pulse at pH 6 (△) and pH 12 (*). Inset: MV^{2+} build up from the reaction of the intermediate with methyl viologen (MV^{2+}) at 605 nm at pH 6.

A similar spectrum is obtained when the pH is raised to 12 where 8AA exists in its deprotonated form. In both the pHs, the spectra were found to undergo a second order decay. The reducing nature of the intermediate is confirmed by the formation of methyl viologen radical
cation (MV") at 605nm at pH 6 (Fig. 3.9), resulting from the reaction of an electron transfer reaction from the reducing intermediate to the oxidant, MV²⁺. A G(MV") (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 H¹ with 8AA at acidic pH (pH 1) with a single maximum at around 330 nm (Fig. 3.10).

![Graph showing transient absorption spectrum](image)

**Figure 3.10:** Reaction of 8AA with H¹: Transient absorption spectrum obtained by pulse radiolysis of a solution containing 8 azaadenine (1 × 10⁻³ mol dm⁻³) at 40 µs after the pulse at pH 1

The spectral features observed from the reaction of e⁻ with 8AA are similar to that of 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 \((k \cong 10^7 \text{ s}^{-1})\)\(^{24-27}\). 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 \(\lambda_{\text{max}}\) 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 \((8\text{AA(NH}^+))\) (Scheme 3.4). A strong evidence for the spin density at carbon came from the quantitative electron transfer from this radical to MV\(^{2+}\) [Fig.3.8] as carbon centered radical can act as an electron donor. Such properties were unambiguously demonstrated using the oxidants MV\(^{2+}\), pNAP (p-nitroacetophenone) \(etc.\) in the case of adenine and its derivatives.\(^{5,24,25}\) However, there are 3 similar sites of electron attack and hence the electron adducts must be represented by their mesomeric structures as shown in the (scheme 3.4). Correspondingly the protonated counterparts would exist in their tautomeric forms (Scheme 3.4). 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\(_2\)PO\(_4^-\) are common in adenine and hypoxanthine derivatives having substitution at their N-9 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 MV²⁺ 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 C-2 and C-8 positions (these are the two potential sites of carbon-protonation) is low due to 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 due to the deprotonation at N-9 position as 8AA has a pKₐ value at around 11.5.

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 analogues 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$^+$. 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. 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 (Figure 3.9) is therefore attributed to the formation of this radical.

\[
\begin{align*}
\text{amine} \quad + \quad \text{H}^* \quad \rightarrow \quad (16) \quad \text{8AA-N}^* (3)
\end{align*}
\]

**Conclusion**

The free radical chemistry of 8AA is important due to its relevance in the therapeutic applications. The reaction of hydroxyl radicals demonstrates an entirely different reaction mechanism compared to that of adenine. The formation of a substituted hydroxyl radical adduct at the C(8) 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\(^-\), SO\(_4\)^-, 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\(^-\) at \(pH = 14\) and of SO\(_4\)^- at basic pH is an interesting observation.
References


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18. The reaction of O^+ is carried out in solution containing 1 mol dm^{-3} sodium hydroxide and therefore the pH is assumed as 14.


