Chapter 5

Redox reactions of thiouracils: A pulse radiolysis and theoretical study
Part A

Oxidation reactions of 2-thiouracil

Abstract

The reactions of \( ^\cdot \text{OH} \) generated via pulse radiolysis with nucleic acid base analogue 2-thiouracil (designated as \( 1 \)) was studied in aqueous medium by optical absorption detection. The transient absorption spectrum is characterized by \( \lambda_{\text{max}} \) at 430 nm at pH 6.5. A bimolecular rate constant, \( k_2 \) of \( 9.6 \times 10^9 \text{ M}^{-1} \text{ s}^{-1} \) was determined for this reaction via competition kinetics. The transient species was assigned as a dimer radical cation \( 2^{\cdot}+ \), formed by the reaction of \( 1 \) with its radical cation \( 1^{\cdot}+ \) and an equilibrium constant of \( 4.7 \times 10^3 \text{ M}^{-1} \) was determined for the dimerization. The reactions of \( 1 \) with pulse radiolytically produced \( \text{Br}_2^{\cdot} \) at pH 6.5 as well as \( \text{Cl}_2^{\cdot} \) at pH 1 has produced transient with \( \lambda_{\text{max}} \) at 430 nm. DFT calculations at BHandHLYP/6-311+G(d,p) level showed that \( 2^{\cdot}+ \) is characterized by a 2c-3e sulphur-sulphur bond, \( [-\text{S} : : \text{S}^-]^{\cdot} \). Using the TDDFT calculations the \( \lambda_{\text{max}} \) of \( 2^{\cdot}+ \) is predicted as 420 nm in aqueous medium. The peak was characteristic of the 2c-3e bond due to \( 2\sigma \rightarrow 1\sigma^* \) electronic excitation. \( 2^{\cdot}+ \) can be considered as the first reported example of a dimer radical cation in pyrimidine heterocyclic system. Further, 5- and 6- substituted (substituents are \(-\text{F}, -\text{Cl}, -\text{NH}_2, -\text{N(CH}_3)_2, -\text{OCH}_3, -\text{CF}_3, -\text{CH}_3, -\text{CH}_2\text{CH}_3, \text{n-propyl, phenyl, and benzyl}) and 5, 6- substituted (both symmetrically and randomly substituted) 2-thiouracil dimer radical cations have been characterized by theoretical calculations.
Publications from this section


5.1 Introduction

2-thiouracil (designated as \textbf{1}), a minor component of natural t-RNA is chemically the thio analogue of RNA base uracil (U) (Figure 5.1). Derivatives of \textbf{1} have been used as melanoma (tumor derived from melanin forming cells) seekers as they bind covalently to the growing melanin polymer through sulphur. Furthermore, derivatives of \textbf{1} have been used for early diagnosis as well as chemotherapy of disseminated melanoma\textsuperscript{2-6}. \textbf{1} and its several derivatives, for example, 6-methyl-2-thiouracil, 6-n-propyl-2-thiouracil and 6-phenyl-2-thiouracil are effective for the treatment of hyperthyroidism\textsuperscript{7}. Bhabak and Mugesh have shown that the antioxidant activity of 6-n-propyl-2-thiouracil and 6-methyl-2-thiouracil can be attributed to the inhibition of peroxynitrite-mediated nitration of tyrosine residues in proteins\textsuperscript{8}. \textbf{1} is also reported\textsuperscript{9} as a selective inhibitor of neuronal nitric oxide synthase, an enzyme that catalyses the NADPH-dependent formation of nitric oxide from L-arginine and O\textsubscript{2}. Among the several tautomeric structures possible for \textbf{1}, the experimental and theoretical studies suggest that the oxo-thione form (Figure 5.1) is the most stable tautomer\textsuperscript{10-15}.

\textbf{Figure 5.1:} Structures of uracil (U), 2-thiouracil (\textbf{1}) and thiourea.

The one electron oxidation reactions of thioethers, thioamines, thioacids, methionine and its derivatives induced by specific one electron oxidants such as \textsuperscript{1}OH or SO\textsubscript{4}\textsuperscript{2-}, and dihalogen radical ion (X\textsubscript{2}\textsuperscript{•}),
where X = Cl, Br, I) have been extensively studied by pulse radiolysis with optical absorption or conductance detection\textsuperscript{16}. The formation of intra or intermolecular 2-center-3-electron (2c-3e) bonds such as [\(-S\cdot\cdotX\cdot\cdot-\)]\textsuperscript{+} (X = N, O, Cl, Br, or I) or [\(-S\cdot\cdotS\cdot\cdot-\)]\textsuperscript{+} is a well-established reaction demonstrated by pulse radiolysis experiments on sulphur compounds. The 2c-3e bond\textsuperscript{17} represented by the symbol \(\cdot\cdot\) is characterized by two electrons located in a bonding \(\sigma\) orbital and one electron in an antibonding \(\sigma^*\) orbital\textsuperscript{18}. In aryl substituted sulphur compounds, the one electron oxidation gives either a stable monomer radical cation or a neutral radical formed via hydroxylation or proton elimination reaction. In aryl substituted compounds, the usually observed redundancy in dimer radical cation formation is primarily attributed to the spin delocalization inside the aromatic ring and also to the high reactivity of aromatic ring towards \(\cdot\text{OH}\)\textsuperscript{19-22}. Wang et al has studied the reactions of \(\cdot\text{OH}\) with thiourea where the transient observed have a \(\lambda_{\text{max}}\) of 400 nm with a bimolecular rate constant of \(1.2 \times 10^{10}\) M\(^{-1}\) s\(^{-1}\) by competition kinetics\textsuperscript{23}. The intermediate in this reaction was assigned as a dimer radical cation with an [\(-S\cdot\cdotS\cdot\cdot-\)]\textsuperscript{+} 2c-3e bond formed via the reversible combination of a primary radical cation to another ground state molecule of thiourea.

To the best of our knowledge, no work has been published on the reactions of one electron oxidants with \(\mathbf{1}\) and we expect that due to the structural similarity between \(\mathbf{1}\) and thiourea around the S=C bond (see Figure 5.1, colored region), the reactivity of \(\mathbf{1}\) may show some similarity to that of thiourea. Apart from this reason, \(\mathbf{1}\) is a worthy molecule to study using pulse radiolysis because little is known about its fate in living systems in the presence of large amount of ROS such as \(\cdot\text{OH}\) generated from endogenous cellular metabolism and external ionizing radiations. Therefore, its in-vitro study may throw some light into the
chemical basis of such interactions. Hence, herein we focus on the mechanistic features of the reaction of 1 with \(^{1}\)OH, and compared the transients derived from the reactions of specific one electron oxidants such as Br\(_2\)^\(\cdot\) and Cl\(_2\)^\(\cdot\) with 1. Pulse radiolysis technique in conjunction with optical absorption detection method is used for this study to detect the transient species formed in the reaction in aqueous medium. Further, DFT level theoretical calculations have been used to substantiate the experimental results as well as to look at various possibilities.

5.2 Theoretical details

Theoretical calculations were carried out by means of BHandHLYP density functional method\(^{24}\) using the 6-311+G(d,p) basis set. The optimizations of the structures at BHandHLYP/6-311+G(d,p) level in gas phase were followed by frequency calculations at the same level of theory. The solvent effects were taken into account in aqueous phase optimizations by means of the polarizable continuum model (PCM)\(^{25}\). The charge and spin density distributions in transient systems were considered by using the Mulliken scheme. The S-S bond dissociation energies in [\(-S:\cdot:S-\)] bonded dimer spices were calculated as, BDE = E[dimer radical cation] – E[(neutral molecule + radical cation)]. Time dependent DFT (TDDFT)\(^{26}\) calculations at BHandHLYP/6-311+G(d,p) level of theory in the solution phase were done to explore the electronic excitations in transient species. Electronic structure calculations were performed by using Gaussian 09 suite of programs\(^{27}\).

5.3 Results and discussions

A. Theoretical studies

All the plausible reactions that \(^{1}\)OH can undergo with 1 are initially deduced and are depicted in Figure 5.2. The intermediate
radicals obtained are respectively (i) $1^{**}$ (radical cation) by the one electron oxidation, (ii) $3^*$ (C5_OH$^*$ adduct), $4^*$ (C6_OH$^*$ adduct), and $5^*$ (S8_OH$^*$ adduct) via adduct formations and (iii) $6^*$ ((N1-H)$^*$, the H7 -abstracted radical) and $7^*$ ((N3-H)$^*$, the H9-abstracted radical) by H-abstraction reactions. Transients $3^*$, $4^*$, and $5^*$ can also be obtained by the hydroxylation of transient $1^{**}$ by water molecules; similarly, transients $6$ and $7$ can be obtained by the deprotonation reactions of $1^{**}$.

![Figure 5.2: Schematic of possible reactions that *OH can induce in 2-thiouracil](image)

The dominant reactions of *OH with pyrimidine nucleobases are addition to the C5C6 double bonds (for example in cytosine, uracil and thymine) and to a lesser extent, the H-abstraction from the methyl group of thymine$^{28}$. The H-abstractions as well as one electron oxidation
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reactions are not competitive as *OH additions in natural nucleobases. The presence of sulphur in 1 can rationalize our proposition of one electron oxidation in the present case. The H-abstractions from C5 and C6 atoms are also possible; however, the C-H bond energy is comparatively higher than the N-H bond energy.

Depicted in Figure 5.3a is the solution phase optimized geometry of 1 at BHandHLYP/6-311+G(d,p) level of theory along with its HOMO (Figure 5.3b). For a comparison the HOMO of uracil (Figure 5.3c) at the same level of theory is also presented. A recent theoretical study demonstrated\textsuperscript{29} that the *OH interaction with C5 atom (main donor for the HOMO) of uracil is a kinetically driven process; which accounts for the transient produced during the pulse radiolysis\textsuperscript{30}. We have observed a planar geometry for 1 with C5-C6 bond as the shortest bond (1.334 Å) of the ring. The HOMO of 1 is more localized about the S=C bond. Accordingly, the principal interaction of *OH should be with the C2=S8 unit or more precisely with the S8-atom based on HOMO. Hence, by exploring the optimized geometry and HOMO one can consider the nucleophilic centers in 1 for an electrophilic reagent such as *OH as C5, C6 and S8 atoms.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.3.png}
\caption{(a) Optimized geometry of 2-thiouracil in solution phase, bond lengths are in Å units (b) and (c) are HOMO of 2-thiouracil and uracil respectively (isocontour value = 0.05 a.u.)}
\end{figure}
The ionization potential (IP) of 1 (computed as the difference in energy between 1 and its radical cation $1^{\ddagger}$ at their respective optimized geometries) at BHandHLYP/6-311+G(d,p) level of theory in solution phase was 6.39 eV which is slightly higher than the calculated IP value 6.0 eV for thiourea at the same level of theory. Previously reported theoretical IP value of thiourea was 6.12 eV computed at BHandHLYP/6-31+G(d,p) level of theory in aqueous medium\textsuperscript{31}. The solution phase optimized geometry of radical cation $1^{\ddagger}$ is depicted in Figure 5.4. The spin density of $1^{\ddagger}$ is fully localized over the sulphur atom and the positive charge is dispersed to the ring and maximum is on the C2 atom (0.408 a.u.). Substantial changes in the ring bond lengths (except C5C6 bond) and C2S8 bond has been observed for 1 upon ionization. Marked elongation respectively 0.074 Å and 0.062 Å occurs for the C2S8 and C2N3 bonds while noticeable contraction (by 0.039 Å) occurs for the C2N1 bond in $1^{\ddagger}$ in comparison to 1.

The optimized structures of the $\cdot$OH addition products are presented in Figure 5.4 with selected geometrical parameters. The addition reactions of $\cdot$OH at C5, C6 and S8 of 1 occurs without the intervention of any reactant complexes, which means addition processes are barrier free. The atomic spin density of $3'$ is found maximum on the C6 atom, while for $4'$ the spin density is localized on the adjacent C5 atom. Considerable elongation of the C5C6 bond occurs upon $\cdot$OH additions on C5 or C6. Slight puckering of the ring occurs at the addition sites for both C5 and C6 additions. The spin density of $5'$ is more dispersed, the maximum values are found at the C2 and C5 atoms. It has been found that, $3'$ represent the thermodynamically most stable among the three $\cdot$OH addition products. The $4'$ and $5'$ are respectively by 9.53 kcal/mol and 33.13 kcal/mol less stable than $3'$ in solution phase.
The H-abstraction from N/N3 atom results in the formation of H-abstracted radicals $6^* / 7^*$ and a water molecule. The optimized structures of H-abstracted radicals $6^*$ and $7^*$ are depicted in Figure 5.4. The atomic spin density respectively 1.174 a.u. and 1.168 a.u. are localized on the S8 atom in both $6^*$ and $7^*$. A possible rationale for the spin localization on the exocyclic S-atom can be the increased aromatic character of the ring moiety by pushing the odd electron out of the ring. The $6^*$ is found to be more stable than $7^*$ by an amount 2.57 kcal/mol.

**Figure 5.4:** Solution phase optimized geometries of transients. Selected bond lengths are in Å units. Mulliken spin density maximum located on each are given in parenthesis.

The H-abstraction reactions are assumed to take place from the reactant complexes 1…*OH$_1$(1) (for the H7 abstraction) and 1…*OH$_2$(2) (for the H9 abstraction) as depicted in Figure 5.5. Both the reactant complexes are stabilized by means of moderately strong hydrogen
bonds. Moreover the odd spin on the \(^*\)OH is transferred to the ring moiety both in \(1\ldots^*\)OH\(_{(1)}\) and \(1\ldots^*\)OH\(_{(2)}\). Compared to the separate entities \(1\ldots^*\)OH, the \(1\ldots^*\)OH\(_{(1)}\) is stabilized by 7.55 kcal/mol while \(1\ldots^*\)OH\(_{(2)}\) is stabilized by 7.01 kcal/mol. The H-abstraction reactions are postulated to occur by the following pathways.

\[
\begin{align*}
1\ldots^*\text{OH}_{(1)} & \rightarrow TS_1 \rightarrow 6^*\ldots\text{H}_2\text{O} & (5.1) \\
1\ldots^*\text{OH}_{(2)} & \rightarrow TS_2 \rightarrow 7^*\ldots\text{H}_2\text{O} & (5.2)
\end{align*}
\]

The product complexes \(6^*\ldots\text{H}_2\text{O}\) and \(7^*\ldots\text{H}_2\text{O}\) are formed from the reactant complexes \(1\ldots^*\)OH\(_{(1)}\) and \(1\ldots^*\)OH\(_{(2)}\) via the transition states \(\text{TS}_1\) and \(\text{TS}_2\). The transition states were located by means of Synchronous Transit-Guided Quasi-Newton (QST3)\(^{32,33}\) search method. Compared to \(1\ldots^*\)OH\(_{(1)}\) and \(1\ldots^*\)OH\(_{(2)}\) unpaired spin is more drained away from \(^*\)OH to the ring structures of both \(\text{TS}_1\) and \(\text{TS}_2\). The vibrational frequency with which the H7 oscillate between N1 and oxygen of the \(^*\)OH in \(\text{TS}_1\) is \(-1326.74\) cm\(^{-1}\); the corresponding value for the oscillation of H9 between N3 and oxygen of the \(^*\)OH in \(\text{TS}_2\) is \(-1393.81\) cm\(^{-1}\). In the product complex \(6^*\ldots\text{H}_2\text{O}\), the unpaired spin is delocalized on the ring with maximum at S8 (0.666 a.u.) and C5 (0.367 a.u.) atoms. However for the \(7^*\ldots\text{H}_2\text{O}\) complex, the unpaired spin is localized fully on S8 atom (1.196 a.u.). Later, the product complexes separate into the H-abstracted radical and a water molecule. The optimized structures of the H-abstraction pathways (as shown by equations 5.1 and 5.2) are depicted in Figure 5.5.
The relative energies (relative with respect to separated entities 1 and *OH) for the H-abstractions are given in Figure 5.6. The activation energy ($E_{act}$) for the H7 abstraction from N1 is 3.04 kcal/mol; while $E_{act}$ for the H9 abstraction is 3.99 kcal/mol. Hence, the H7 abstraction is found to be thermodynamically somewhat more favorable than the H9 abstraction. The product complex 6*…H2O is found to be more stable than 7*…H2O by 4.61 kcal/mol.
Chapter 5

TDDFT calculations

The calculated $\lambda_{\text{max}}$ values and oscillator strengths for the transients considered herein using the BHandHLYP/6-311+G(d,p) TDDFT method in solution phase is compiled in Table 5.1.

Table 5.1: The $\lambda_{\text{max}}$ values and oscillator strengths of transients.

<table>
<thead>
<tr>
<th>Transient</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1*</td>
<td>228</td>
<td>0.234</td>
</tr>
<tr>
<td>3*</td>
<td>337</td>
<td>0.252</td>
</tr>
<tr>
<td>4*</td>
<td>227</td>
<td>0.352</td>
</tr>
<tr>
<td>5*</td>
<td>340</td>
<td>0.183</td>
</tr>
<tr>
<td>6*</td>
<td>242</td>
<td>0.013</td>
</tr>
<tr>
<td>7*</td>
<td>245</td>
<td>0.318</td>
</tr>
</tbody>
</table>

Figure 5.6: Relative energy profile of the H-abstraction reactions in aqueous phase computed at BHandHLYP/6-311+G(d,p) level of theory.
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It can be seen from the table that $\lambda_{\text{max}}$ values of $1^\ast$, $4^\ast$, $6^\ast$, and $7^\ast$ falls in the middle UV region while for $3^\ast$ and $5^\ast$ lies in the visible region.

The results of these theoretical studies have provided information about the reaction feasibilities and it was found that, (i) IP of $1$ being comparable to the IP of thiourea, favorable for a one electron oxidation reaction, (ii) the $^\ast \text{OH}$ addition reactions are barrierless processes, (iii) the H-abstractions are having sufficient activation energy barriers and (iv) the TDDFT calculations gave the hint of $\lambda_{\text{max}}$ of the transients. Based on the above theoretical results in hand we have performed the pulse radiolysis experiments. We have considered the optical absorptions of the transients from 300 nm to 650 nm which is the generally used pulse radiolysis wavelength range.

**Pulse radiolysis studies**

The transient absorption spectrum obtained from the reaction of $^\ast \text{OH}$ with $1$ at 5 $\mu$s after the pulse is characterized by a $\lambda_{\text{max}}$ 430 nm at pH 6.5 (Figure 5.7). The time resolved spectra didn’t exhibit any change in $\lambda_{\text{max}}$ except a decrease in intensity at higher time scales. For $\text{N}_2\text{O}$ saturated solutions, the effective radiation chemical yield of $^\ast \text{OH}$ ($G(^\ast \text{OH})$) converting a given substrate ($S$) into substrate-derived radicals can be calculated on the basis of the formula 5.3 reported by Schuler et al.\(^{34}\), where $k_{(S)}$ represent the pseudo-first-order rate constant of $^\ast \text{OH}$ reaction with $S$ and $4.7 \times 10^8$ s$^{-1}$ is the track recombination frequency.

$$G(^\ast \text{OH}) = 5.2 + 3 \frac{[k_{(S)}/(4.7 \times 10^8)]^{1/3}}{1 + [k_{(S)}/(4.7 \times 10^8)]^{1/3}}$$ (5.3)

The $G(^\ast \text{OH})$ for the reaction of $^\ast \text{OH}$ with $1$ is thus determined as $5.46 \times 10^{-7}$ M J$^{-1}$ using a pseudo-first-order rate constant of $4.16 \times 10^6$ s$^{-1}$ at 1 mM concentration of $1$. It means that ca. 98% of the $^\ast \text{OH}$
produced (total yield is considered as $5.6 \times 10^{-7}$ M J$^{-1}$) during the pulse irradiation is reacting with 1 leading to transients.

**Figure 5.7:** Transient absorption spectrum recorded at (■) 5 µs after pulse irradiation of an N$_2$O-saturated aqueous solution containing 2-thiouracil (1 mM) at pH 6.5 (Dose = 13.7 Gy/pulse). Insets: (i) Time vs. absorbance of the transient at 430 nm (ii) competition kinetics of *OH reactions with 2-propanol (0.05 M) and 2-thiouracil (0.5 to 5 mM).

The bimolecular rate constant for *OH reaction with 1 was determined by competition kinetics using 2-propanol. Competition kinetics studies were performed at a constant concentration of 2-propanol (0.05 M) containing various concentrations of 1 (0.5 to 5mM). The two competing reactions (equations 5.4 and 5.5) give an expression represented by equation 5.6, where $A_0$ and A are the absorbance noted at 430 nm in the absence and presence of 2-propanol.

\[ \cdot \text{OH} + (\text{CH}_3)_2\text{CHOH} \rightarrow (\text{CH}_3)_2\cdot \text{COH} + \text{H}_2\text{O} \quad \text{(5.4)} \]

\[ \cdot \text{OH} + \text{2-thiouracil} \rightarrow \text{Transients} \quad \text{(5.5)} \]
Using the literature\textsuperscript{35} value of $k(2\text{-propanol}^*\text{OH})$ as $1.9 \times 10^9$ M$^{-1}$ s$^{-1}$, the bimolecular rate constant ($k(2\text{-thiouacil}^*\text{OH})$) obtained from the slope of the plot (inset (ii) in Figure 5.7) was $9.6 \times 10^9$ M$^{-1}$ s$^{-1}$.

In principle none of the predicted transients (Figure 5.2) seems to be responsible for the experimentally observed transient absorption spectrum. Being electrophilic in nature, $^*\text{OH}$ has a high chance of attacking the sulfur center (see Figure 5.3 b) thereby producing a radical cation $1^*$. However, an absorption maximum of 228 nm is predicted for this radical cation by using TDDFT calculation (Table 5.1). Hence, we assumed the probability of certain secondary transient such as dimer formation by making use of the initially formed $1^*$ on reaction with $1$. Therefore, the possibility of a dimer radical cation formation $2^*$ (see Figure 5.8) has been considered similar to the $^*\text{OH}$ reaction with thiourea\textsuperscript{23}.

\[
\frac{A_t}{A} = 1 + \frac{k(2\text{-propanol}^*\text{OH})}{k(2\text{-thiouacil}^*\text{OH})} \frac{[2\text{-propanol}]}{[2\text{-thiouacil}]} \quad (5.6)
\]

\textbf{Figure 5.8:} Schematic of dimer radical cation formation from $1^*$ and $1$.

In order to look at this prediction, concentration dependent intensity of absorption at 430 nm was investigated. This experiment revealed that the transient absorption intensity at 430 nm increases with increase in the concentration of $1$ (Figure 5.9). The equilibrium constant $K$ for the reversible reaction depicted in Figure 5.8 is calculated by
means of equation (5.7) as used by Wang et al.\(^\text{23}\) where \(A_0\) is the transient absorbance from pulse radiolysis of 2 mM solution of 1, and A is the transient absorbance at a given concentration of 1.

\[
A_0/A = 1 + K^{-1}[\text{2-thiouracil}]^{-1}
\] (5.7)

The equilibrium constant is determined by taking the inverse of the slope of the plot \((A_0/A)-1\) vs. \([\text{2-thiouracil}])^{-1}\) as shown in Figure 5.9.

![Figure 5.9: \((A_0/A)-1\) against \([\text{2-thiouracil}])^{-1}\) plot based on equation (5.7). Inset (i): The variation of absorbance of the transient with \([\text{2-thiouracil}])\) at 430 nm.](image)

The K value obtained from the linear plot is \(4.7 \times 10^3\, M^{-1}\), which is smaller than the value reported\(^\text{23}\) for thiourea \((5.5 \times 10^5\, M^{-1})\) and tetramethylthiourea \((7.6 \times 10^4\, M^{-1})\) and selenourea \((7.9 \times 10^4\, M^{-1})\) \(^\text{31}\). However, lower K values were reported for dimer radical cation formations in n-allyl thiourea \((4.9 \times 10^3\, M^{-1})\) \(^\text{36}\), methionine \((1.9 \times 10^3\, M^{-1})\) \(^\text{37}\) and its methyl ester \((3.1 \times 10^3\, M^{-1})\)\(^\text{38}\) and selenomethionine \((9.2 \times 10^3\, M^{-1})\)\(^\text{37}\). Therefore, it can be well assumed that a dimer radical
of the type $2^{*+}$ is the major intermediate herein. More experimental evidence for the formation of dimer radical cation is obtained by studying the reaction of $\text{Br}_2^{*-}$ and $\text{Cl}_2^{*-}$ with $1$. The reaction of $\text{Br}_2^{*-}$ with $1$ is studied at pH 6.5 and the ensuing absorption spectrum (Figure 5.10) is characterized with a $\lambda_{\text{max}}$ at 430 nm and the spectrum is found to be very similar to the $^\bullet \text{OH}$ reaction spectrum (at pH 6.5). Moreover, the absorbance of the transient has increased with increase in the concentration of $1$ as in the case of the reaction of $^\bullet \text{OH}$. The bimolecular rate constant is determined from the slope of the plot of $k_{\text{obs}}$ against varying concentrations of $1$ at the absorption maximum of $\text{Br}_2^{*-}$ ($\lambda_{\text{max}}$ 360 nm) and is $2.1 \times 10^9 \text{ M}^{-1} \text{s}^{-1}$.

Figure 5.10: Transient absorption spectrum obtained in pulse irradiation of N$_2$O-saturated aqueous solution containing KBr (0.01M) and 1 mM 2-thiouracil at 5 µs after the pulse at pH 6.5 (Dose = 11.8 Gy/pulse). Inset: (i) [2-thiouracil] vs. absorbance of the transient at 430 nm (ii) Plot of $k_{\text{obs}}$ vs. varying concentrations of 2-thiouracil measured at 360 nm.
The reaction of Cl$_2^\cdot$ with 1 is investigated in aerated aqueous solutions at pH 1. The transient absorption spectrum is characterized by $\lambda_{\text{max}}$ at 430 nm (Figure 5.11). The spectrum is identical in shape to that resulting from *OH and Br$_2^\cdot$ (both at pH 6.5), apart from a better G × ε value observed for the Cl$_2^\cdot$ reaction. The bimolecular rate constant for the reaction is determined at the characteristic absorption maximum of Cl$_2^\cdot$ ($\lambda_{\text{max}} = 345$ nm). The slope of the plot of $k_{\text{obs}}$ against concentration of 1 (inset (ii) in Figure 5.11) at 345 nm gave a value of $3.2 \times 10^9$ M$^{-1}$ s$^{-1}$ as the second order rate constant. This rate constant is two orders of magnitude higher than the earlier reported rate constant of $3.5 \times 10^7$ M$^{-1}$ s$^{-1}$ by Patterson et al.$^{39}$ for Cl$_2^\cdot$ reactions with uracil.

**Figure 5.11:** Transient absorption spectrum obtained in pulse irradiation of an aqueous solution containing KCl (0.01 M) and 1 mM 2-thiouracil at 5 $\mu$s after the pulse at pH 1 (Dose = 19.3 Gy/pulse). Inset: (i) Dependence of absorbance of transient with [2-thiouracil] at 430 nm. (ii) Plot of $k_{\text{obs}}$ vs. varying concentrations of 2-thiouracil measured at 345 nm.
Comparable absorption spectrums obtained for the $^\cdot$OH, Br$_2$\textsuperscript{•–} and Cl$_2$\textsuperscript{•–} reactions with 1 indicate the formation of identical transient species. However, if one closely looks at the spectrum resulting from $^\cdot$OH, there is a small hike in the 340 nm region (Figure 5.7). Although this hike is not very significant, one can assume that there could be a small contribution from the OH-adduct radicals as the TDDFT calculation predicts the absorption maximum at 337 nm and 340 nm for 3\textsuperscript{•} and 5\textsuperscript{•}.

**Theoretical studies on dimer radical cation**

The geometry of 2\textsuperscript{•+} is presented in Figure 5.12 along with selected bond length parameters. The unpaired spin is evenly distributed on the S atoms. The S-S bond length is 2.797 Å and other bond lengths are equal for both the 2-thiouracil units, suggesting a symmetrical interaction of the two molecules. The S-S bond in this dimer system is [−S···S−]\textsuperscript{+} type 2c-3e bond. TDDFT calculation shows that this transient absorbs at 420 nm and corresponds to transition from the HOMO $\sigma$ orbital to the LUMO $\sigma^*$ orbital (Figure 5.13). The $\lambda_{\text{max}}$ obtained in the calculation is in good agreement with the experimental value of 430 nm which further validates the assumption that the transient species is a dimer radical cation.

![Figure 5.12 Optimized geometry of dimer radical cation of 2-thiouracil. Bond lengths are in Å units. Mulliken spin densities are given in parenthesis.](image)
Further validation of the theoretical results has been obtained by modeling dimer radical cations of thiourea, tetramethylthiourea, and selenourea. The one electron oxidations of these compounds by pulse radiolysis experiments have earlier established the formations of dimer radical cations\textsuperscript{23,31}. For these systems, the computed bond distances, Mulliken spin densities, BDE, and $\lambda_{\text{max}}$ at BHandHLYP/6-311+G(d,p) level in aqueous medium is compiled in Table 5.2.

Among the sulphur based dimer radical cations, the S-S bond distance is the maximum for thiourea dimer whereas $2^+$ has shown the minimum value. The unpaired spin is evenly distributed in the interacting S or Se atoms of the dimer radical cations. The maximum spin density is observed on $2^+$. The BDE is found maximum for selenourea and the least for tetramethylthiourea. The $\lambda_{\text{max}}$ of the dimer cations.

Figure 5.13: Theoretically calculated absorption spectrum of $2^+$ due to electron transition from $\sigma$ to $\sigma^*$. 

![Figure 5.13: Theoretically calculated absorption spectrum of $2^+$ due to electron transition from $\sigma$ to $\sigma^*$.]
radical cations of thiourea and selenourea are in good agreement with the earlier reported values.

Table 5.2: The Mulliken spin densities, X-X bond distance, BDE of S-S (or Se-Se) bond, \( \lambda_{\text{max}} \) and oscillator strength \( f \) of dimer radical cations of thiourea, tetramethylthiourea, selenourea and 2-thiouracil.

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Dimer radical cation of</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>thiourea</td>
<td>tetramethyl thiourea</td>
<td>selenourea</td>
<td>2-thiouracil</td>
</tr>
<tr>
<td>Spin density (a. u.) on X</td>
<td>0.502 (0.470(^{\text{§}}))</td>
<td>0.547</td>
<td>0.520 (0.472(^{\text{§}}))</td>
<td>0.613</td>
</tr>
<tr>
<td>Bond length (Å): X-X</td>
<td>2.806 (2.808(^{\text{§}}))</td>
<td>2.802</td>
<td>2.999 (2.975(^{\text{§}}))</td>
<td>2.797</td>
</tr>
<tr>
<td>BDE (kcal/mol)</td>
<td>16.13 (20.53(^{\text{§}}))</td>
<td>11.24</td>
<td>17.34 (21.14(^{\text{§}}))</td>
<td>15.31</td>
</tr>
<tr>
<td>( \lambda_{\text{max}} ) (nm)</td>
<td>415</td>
<td>467</td>
<td>415 (415(^{\text{§}}))</td>
<td>420</td>
</tr>
<tr>
<td>( f )</td>
<td>0.396</td>
<td>0.339</td>
<td>0.386</td>
<td>0.404</td>
</tr>
<tr>
<td>Experimental ( \lambda_{\text{max}} ) (nm)</td>
<td>400(^{\text{§}})</td>
<td>450(^{\text{§}})</td>
<td>410(^{\text{§}})</td>
<td>430</td>
</tr>
</tbody>
</table>

\(^{\text{§}}\)X = S, \(^{\text{§}}\)X = Se. \(^{\text{§}}\)Spin density on X of monomer radical cation, \(^{\text{§}}\) from reference 33, \(^{*}\)from reference 23.

Several substituted 2-thiouracils have been reported to be clinically useful systems as antioxidants and anticancer agents\(^{8,40-45}\). Since theoretical data provide accurate description of the transients produced due to reactions with ROS, it is felt that theoretical characterization of dimer radical cations of substituted 2-thiouracils will be useful for conducting future experiments. In Table 5.3, BDE, spin density, \( \lambda_{\text{max}} \), oscillator strength and structural data of 33 dimer radical cations of substituted 2-thiouracil systems are provided and among them 11 are 5-C substituted, 11 are 6-C substituted and the rest are substituted at both 5- and 6-C positions. The selected substituents are –F, –Cl, –NH\(_2\),
–N(CH$_3$)$_2$, –OCH$_3$, -CF$_3$, -CH$_3$, -CH$_2$CH$_3$, n-propyl, phenyl, and benzyl. The S-S bond length is almost consistent with all the substituted systems and also the $\lambda_{\text{max}}$ values of substituted dimer radical cations are close to the $\lambda_{\text{max}}$ of parent 2** molecule.

Compared to the parent 2** system, the substitution does not make any substantial change in the S-S bond distances as well as spin distributions. With NH$_2$ substitution at 5-C position, the radical cation becomes unstable due to negative BDE. Same is the case with N(CH$_3$)$_2$ substitution at 5-C and 6-C positions. In the case of 6-substituted 2-thiouracils (except the Cl and CF$_3$ substituted systems), more spin density on the S atoms is observed when compared to the parent 2** molecule. The NH$_2$, F, OCH$_3$, and N(CH$_3$)$_2$, substituted systems show an increase in spin density on S atoms than their corresponding 5-substituted systems. The spin densities of the chlorine substituted thiouracil are almost invariable in 5- and 6- substituted cases. There is decline in the spin values for CF$_3$, n-alkyl, phenyl, and benzyl substituted in comparison to their corresponding 5-substituted counterparts.

The BDE’s of all the 6-derivatives are higher than the 5-substituted systems; notable changes have been observed with NH$_2$, OCH$_3$, N(CH$_3$)$_2$, and phenyl derivates. In the 5, 6- disubstituted cases, eight symmetrically substituted (NH$_2$, F, OCH$_3$, Cl, N(CH$_3$)$_2$, CF$_3$, CH$_3$, CH$_2$CH$_2$CH$_3$) and three mixed combinations (5-CH$_3$ & 6-N(CH$_3$)$_2$, 5-CH$_2$CH$_3$ & 6-N(CH$_3$)$_2$, and 5-CF$_3$ & 6-N(CH$_3$)$_2$).
Redox reactions of thiouracils.....

Table 5.3: The Mulliken spin densities, S-S bond distance, BDE, $\lambda_{\text{max}}$ and oscillator strength ($f$) of 33 substituted dimer radical cations.

<table>
<thead>
<tr>
<th>Substituent position at</th>
<th>Spin density on S of radical cation of</th>
<th>S-S bond length (Å)</th>
<th>BDE (kcal/mol)</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$f$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Monomer</td>
<td>Dimer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NH$_2$</td>
<td>0.258</td>
<td>0.067</td>
<td>2.794</td>
<td>-1.50</td>
<td>420</td>
</tr>
<tr>
<td>F</td>
<td>1.161</td>
<td>0.610</td>
<td>2.796</td>
<td>15.14</td>
<td>420</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>0.334</td>
<td>0.610</td>
<td>2.797</td>
<td>8.70</td>
<td>422</td>
</tr>
<tr>
<td>Cl</td>
<td>0.754</td>
<td>0.606</td>
<td>2.801</td>
<td>14.99</td>
<td>425</td>
</tr>
<tr>
<td>N(CH$_3$)$_2$</td>
<td>1.139</td>
<td>0.630</td>
<td>2.798</td>
<td>9.82</td>
<td>426</td>
</tr>
<tr>
<td>CF$_3$</td>
<td>1.164</td>
<td>0.634</td>
<td>2.800</td>
<td>14.89</td>
<td>428</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>0.751</td>
<td>0.635</td>
<td>2.797</td>
<td>14.59</td>
<td>420</td>
</tr>
<tr>
<td>CH$_2$CH$_3$</td>
<td>0.752</td>
<td>0.640</td>
<td>2.797</td>
<td>14.70</td>
<td>421</td>
</tr>
<tr>
<td>CH$_3$CH$_2$CH$_3$</td>
<td>0.749</td>
<td>0.650</td>
<td>2.800</td>
<td>14.84</td>
<td>423</td>
</tr>
<tr>
<td>CH$_3$N$_2$</td>
<td>0.215</td>
<td>0.638</td>
<td>2.790</td>
<td>10.03</td>
<td>424</td>
</tr>
<tr>
<td>CH$_3$O$_2$</td>
<td>0.753</td>
<td>0.659</td>
<td>2.801</td>
<td>14.77</td>
<td>424</td>
</tr>
<tr>
<td>H</td>
<td>NH$_2$</td>
<td>1.181</td>
<td>0.631</td>
<td>2.795</td>
<td>15.36</td>
</tr>
<tr>
<td>H</td>
<td>OCH$_3$</td>
<td>1.176</td>
<td>0.629</td>
<td>2.795</td>
<td>15.20</td>
</tr>
<tr>
<td>H</td>
<td>Cl</td>
<td>1.167</td>
<td>0.605</td>
<td>2.782</td>
<td>14.85</td>
</tr>
<tr>
<td>H</td>
<td>N(CH$_3$)$_2$</td>
<td>1.168</td>
<td>0.646</td>
<td>2.795</td>
<td>15.56</td>
</tr>
<tr>
<td>H</td>
<td>CF$_3$</td>
<td>1.199</td>
<td>0.613</td>
<td>2.791</td>
<td>14.96</td>
</tr>
<tr>
<td>H</td>
<td>CH$_3$</td>
<td>1.159</td>
<td>0.627</td>
<td>2.797</td>
<td>15.27</td>
</tr>
<tr>
<td>H</td>
<td>CH$_2$CH$_3$</td>
<td>1.147</td>
<td>0.622</td>
<td>2.793</td>
<td>15.42</td>
</tr>
<tr>
<td>H</td>
<td>CH$_3$CH$_2$CH$_3$</td>
<td>1.143</td>
<td>0.630</td>
<td>2.798</td>
<td>15.44</td>
</tr>
<tr>
<td>H</td>
<td>CH$_3$N$_2$</td>
<td>1.165</td>
<td>0.624</td>
<td>2.797</td>
<td>19.67</td>
</tr>
<tr>
<td>H</td>
<td>CH$_2$O$_2$</td>
<td>1.169</td>
<td>0.640</td>
<td>2.796</td>
<td>15.39</td>
</tr>
<tr>
<td>NH$_2$</td>
<td>NH$_2$</td>
<td>0.097</td>
<td>0.622</td>
<td>2.794</td>
<td>-10.91</td>
</tr>
<tr>
<td>F</td>
<td>F</td>
<td>1.171</td>
<td>0.619</td>
<td>2.791</td>
<td>14.76</td>
</tr>
<tr>
<td>OCH$_3$</td>
<td>OCH$_3$</td>
<td>0.141</td>
<td>0.623</td>
<td>2.793</td>
<td>4.31</td>
</tr>
<tr>
<td>Cl</td>
<td>Cl</td>
<td>0.746</td>
<td>0.602</td>
<td>2.795</td>
<td>15.32</td>
</tr>
<tr>
<td>N(CH$_3$)$_2$</td>
<td>N(CH$_3$)$_2$</td>
<td>0.053</td>
<td>0.643</td>
<td>2.796</td>
<td>-16.89</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>CH$_3$</td>
<td>1.172</td>
<td>0.626</td>
<td>2.795</td>
<td>15.69</td>
</tr>
<tr>
<td>CF$_3$</td>
<td>CF$_3$</td>
<td>1.176</td>
<td>0.624</td>
<td>2.793</td>
<td>14.03</td>
</tr>
<tr>
<td>CH$_3$CH$_2$CH$_3$</td>
<td>CH$_3$CH$_2$CH$_3$</td>
<td>0.680</td>
<td>0.667</td>
<td>2.796</td>
<td>14.53</td>
</tr>
<tr>
<td>CF$_3$</td>
<td>N(CH$_3$)$_2$</td>
<td>1.188</td>
<td>0.646</td>
<td>2.796</td>
<td>15.10</td>
</tr>
<tr>
<td>CH$_3$</td>
<td>N(CH$_3$)$_2$</td>
<td>0.055</td>
<td>0.630</td>
<td>2.792</td>
<td>15.75</td>
</tr>
<tr>
<td>CH$_3$CH$_3$</td>
<td>N(CH$_3$)$_2$</td>
<td>1.203</td>
<td>0.644</td>
<td>2.791</td>
<td>2.55</td>
</tr>
</tbody>
</table>
In symmetrical systems, the spin density values are mainly between that of separate 5-substituted and 6-substituted systems. The BDE’s of 5, 6- NH$_2$ and N(CH$_3$)$_2$ systems are very low, showing that stability wise they are weak compared to either the 5-, or 6- substituted congeners. Of the 33 substituted 2-thiouracils, the 6-phenyl-2-thiouracil produces the most stable dimer radical cation while the 5,6-N,N-dimethyl-2-thiouracil dimer radical cation is the least stable.

5.4 Conclusions

The present study has established that the reaction of OH with 1 is quite distinct from that of the natural base uracil. All the possible reaction channels viz. one electron oxidation, adduct formations, and H-abstractions were analyzed initially. Minor contributions to the experimental spectrum due to OH additions at C5 and S8 have been noticed. However, further theoretical studies showed that, the OH induces the formation of a dimer radical cation 2**, characterized by a [-S: :S-]" 2c-3e bond constitute the major reaction. The 2** formation occurs via the combination of an initially formed radical cation 1**, with another neutral molecule of 1. The reactions of 1 with other specific one electron oxidants such as Br$_2$ and Cl$_2$ also pointed out 2 formations in the pulse radiolysis experiments. Computational calculations at BHandHLYP/6-311+G(d,p) level of theory proved that the excitation of an electron from the doubly occupied $\sigma$-bonding molecular orbital to the singly occupied $\sigma^*$-anti bonding molecular orbital is responsible for the observed experimental spectrum at 430 nm. Using 2-thiourcil as paradigmatic, the dimer radical cation formations in a number of 2-thiouracils have been verified. It has been found that, substitution at 6-C produces more stable dimer radical cations when compared to substitution at 5-C position. The dimer radical cations of 6-C substituted 2-thiouracils are estimated to have higher BDEs thereby strong
Redox reactions of thiouracils.....

[−S=O−] 2c-3e bond. The 6-phenyl-2-thiouracil produces the most stable dimer radical cation among 33 dimer systems studied theoretically. It has also been seen that, the substitutions at 5-C or 6-C position doesn’t make changes in the absorption maximum of parent $2^{**}$. All the substituted dimer radical cations absorb around 420 nm.
Part B

Oxidation reactions of 4-thiouracil

Abstract

Pulse radiolysis experiments have been used to study the reactions of *OH with 4-thiouracil (represented as 1) in aqueous medium. The transient absorption spectrum for *OH reactions with 1 is characterized by $\lambda_{\text{max}}$ 460 nm at pH 7. The bimolecular rate constant $k(4\text{-thiouracil} + \cdot \text{OH})\text{ } 1.7 \times 10^{10}\text{ } \text{M}^{-1}\text{ } \text{s}^{-1}$ was determined by competition kinetics using 2-propanol. The transient is envisaged as a dimer radical cation 2, formed via the combination of an initially formed radical cation 1a with another molecule of 1. The formation constant of 2 was determined as $1.8 \times 10^{4}$ M$^{-1}$. The reactions of Br$_2^*$ (at pH 7), Cl$_2^*$ (at pH 1), and N$_3^*$ (at pH 7) with 1 have also produced transient with $\lambda_{\text{max}}$ at 460 nm. DFT studies at BHandHLYP/6-311+G(d,p) level in aqueous phase have showed that 2 is characterized by $2\sigma \pi \sigma^*$ – $S:\cdot S$ bond. The unpaired electron spin density of 2 is evenly distributed over the two S-atoms (0.65 a.u. each). The BDE of $S:\cdot S$ bond is calculated as 12.4 kcal/mol. The predicted $\lambda_{\text{max}}$ 457 nm (oscillator strength, $f = 0.566$) by using the TDDFT method for 2 is in good agreement with experimental $\lambda_{\text{max}}$. An extension of theoretical calculations has used to study the dimer radical cations of 4-thiothymine and 4-thiouridine molecules; 4-thiouridine can form more stable dimer compared to 2, while the stability of 4-thiothymine dimer is roughly half of 2.
Publications from this section

5.5 Introduction

4-thiouracil (represented as 1) is an important thio derivative of nucleic acid base uracil. Its nucleoside, 4-thiouridine is present as a minor component of t-RNA (at position 8 and rarely at 9) in Escherichia coli (E. coli)\textsuperscript{46}. The 1 residue found at a frequency of roughly two nucleotides for every three molecules of t-RNA in E. coli. The presence of sulphur (in position 10, cf. Figure 5.14) of uracil ring dramatically changes the absorption properties; 1 absorbs at UVA region (320-400 nm) while the canonical DNA/RNA bases display end absorptions\textsuperscript{47} at wavelength longer than 300 nm.

![Figure 5.14: Structures of uracil and 4-thiouracil with atomic numbering.](image)

Thio-substituted nucleosides can be incorporated \textit{in vivo} into the RNA of growing cells (via UV induced photo cross-linking) without perturbing the system under study and are valuable tools for probing the structural and functional studies of folded RNA\textsuperscript{48,49,50-53}. Irradiation of several t-RNAs at 335 nm results in intramolecular photoreaction between the 4-thiouridine in position 8 and the cytidine in position 13 of t-RNA. In photochemotherapy, 4-thiothymidine is used as a photo sensitizer, and its incorporation into the DNA of replicating cells and UV irradiation causes targeted tumour cells destructions with minimal damage to normal cells\textsuperscript{54}. In principle 1 can exists in several tautomeric forms (due to protomeric
tautomerism), but several experimental and theoretical studies demonstrated that the thio-keto form (as given in Figure 5.14) is the most stable one in gaseous and aqueous medium \(^{10,55,10,56-58}\) albeit photoinduced conversion of 2-keto-4-thio form to 2-keto-4-thiol form was reported\(^{59}\) in the excited states of 1.

An interesting feature in the pulse radiolysis and laser flash radiolysis studies of one electron oxidations of organic sulphur compounds in contrast to their oxygen congeners is their ability of the former systems to form 2 center 3 electron (2c-3e) bonded species in addition to the normal oxidation products (adduct systems, hydrogen abstracted systems, monomer radical cations etc.) that are more common\(^{16}\). Both intramolecular (if the molecule contains a second S) and intermolecular 2c-3e sulphur-sulphur bonded neutral, cationic and anionic systems are observed with sulphur compounds. In the present work, we focused on the oxidation reactions of 1 by pulse radiolytically generated \(^{\bullet}\)OH radicals in aqueous medium and substantiated the experimental results by means of theoretical calculations. An understanding of reactivity changes caused by substitution of an oxygen (in uracil) by sulfur atom (in 1) with oxidizing agents in aqueous medium is interesting while considering the applications of modified bases/nucleotides as photo probes for the structural and functional studies of RNA, and their derivatives as chromophores of UVA in photochemotherapy.

### 5.6 Results and discussion

#### A. Pulse radiolysis

**Reactions with \(^{\bullet}\)OH:** Figure 5.15 displays the transient absorption spectrum having \(\lambda_{\text{max}}\) at 460 nm obtained for the reaction of \(^{\bullet}\)OH with 1. The ground state absorption maximum of 1 in water is 327 nm; therefore
the ensuing peak in the spectrum is due to the formation of some transient(s). The species decays by second order kinetics without any change in $\lambda_{\text{max}}$. The bimolecular rate constant for $^\bullet$OH reaction with I is determined by competition kinetics method by using 2-propanol (Figure 5.15) as competitor for $^\bullet$OH and the $k_{(4\text{-thiouracil} + ^\bullet\text{OH})}$ evaluated as $1.7 \times 10^{10}$ M$^{-1}$ s$^{-1}$. This value indicates that the $^\bullet$OH reaction with I occurs at diffusion controlled rate.

**Figure 5.15:** Transient spectrum obtained 2 $\mu$s after the pulse radiolysis of an N$_2$O-saturated solution of 4-thiouracil (1 mM) at pH 7 (Dose = 12.4 Gy/Pulse). Inset (i) Decay of the transient at 460 nm. (ii) Competition kinetics of $^\bullet$OH reactions with 2-propanol (0.05 M) and 4-thiouracil (0.5 to 5 mM) at 460 nm.

It is now known from the theoretical studies that the transients derived from pyrimidine nucleobases are mostly due to kinetic control of the $^\bullet$OH reaction rather than the thermodynamic stabilities of resulting transients. As our system under study is a sulphur compound, in addition to the generic $^\bullet$OH reaction centers (C5C6 double bond) there
should pronounced interaction with the sulphur atom. We have considered three possible direct reactions of \( ^{\cdot}\text{OH} \) with 1 namely, one electron oxidation, adduct formations and H-abstractions as depicted in Figure 5.16. The oxidation of 1 by \( ^{\cdot}\text{OH} \) can result the radical cation represented as 1a. If the addition of \( ^{\cdot}\text{OH} \) is electrophilic, then the nucleophilic centers present on 1 are C5, C6 and S10 atoms. The additions of \( ^{\cdot}\text{OH} \) at C5, C6 and S10 leads to adduct systems represented as 3a, 3b and 3c. The abstractions of H7 from N1 and H9 from N3 lead to the formations of H-abstracted radicals, designated as 4a and 4b.

**Figure 5.16:** Schematic of possible reactions of \( ^{\cdot}\text{OH} \) with 4-thiouracil.

The radical cation, 1a may further initiate mainly three secondary reactions. Hydroxylation of 1a by water molecules can lead to formations of hydroxyl adducts. The deprotonation reactions can give
rise to the formations of transients envisaged in the direct H-abstraction reactions. A dimer radical cation formation designated as 2 by combining with another neutral ground state molecule of 1 (see Figure 5.17) is the third probable reaction. Explicitly, the dimer radical cation is presumed to have a \([-S\cdots S-]\) bond with equal spin distribution over the two S-atoms. We have analyzed the possibility of an intermolecular \([-S\cdots S-]\) bonded dimer radical cation formation by considering the possible dependence of absorbance of the transient with increase in the concentration of 1.

\[
\text{Figure 5.17: Schematic of the formation of dimer radical cation from monomer radical cation and ground state molecule.}
\]

It was found that, both the absorbance at 460 nm (see Figure 5.18 (a)) as well as the decay time of the transient increases with increasing the concentration of 1. The formation constant (K) for the probable dimer radical cation formed by the reversible combination of 1a and 1 is calculated by means of equation 5.8. \(A_0\) is the absorbance of transient from 2 mM solution of 1 and A is the transient absorbance at a given concentration of 1.

\[
A_0/A = 1 + K^{-1}[4\text{-thiouracil}]^{-1}
\]

(5.8)

The K value was determined by taking the inverse of the slope of the plot \((A_0/A) - 1\) vs. \([4\text{-thiouracil}]^{-1}\) as shown in Figure 5.18(b). The K value obtained from the linear plot was \(1.8 \times 10^4\) M\(^{-1}\). Fairly high value
of K noted in the present case is indicative of stable dimer radical cation formation in analogy with dimer formation in relatively small acyclic thiourea molecule\textsuperscript{23}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.18}
\caption{Plots of (a) [4-thiouracil] vs. absorbance of the transient at 460 nm and (b) (\(A_0/A\))-1 against [4-thiouracil]\textsuperscript{1} based on equation (5.8).}
\end{figure}

**Reactions with \(\text{Br}_2\star\), \(\text{Cl}_2\star\) and \(\text{N}_3\star\):** In order to get further insight on the above mechanistic aspect, reactions of \(\text{Br}_2\star\), \(\text{Cl}_2\star\) and \(\text{N}_3\star\) with \textbf{1} were carried out. These radicals generally induce one electron oxidations in pyrimidines\textsuperscript{28}. Transient absorption spectrum obtained for the reaction of \(\text{Br}_2\star\) with \textbf{1} at pH 7 is shown in Figure 5.19; the spectrum is characterized with a \(\lambda_{\text{max}}\) of 460 nm and is well comparable to the \(\cdot\text{OH}\) reaction spectrum presented in Figure 5.15. At longer time scales, the intensity of 460 nm peak decreases while keeping the spectral shapes intact.

The bimolecular rate constant for the reactions of \textbf{1} with \(\text{Br}_2\star\), determined from the concentration dependent pseudo-first-order rate constant at the absorption maximum of the \(\text{Br}_2\star\) (\(\lambda_{\text{max}} = 360\) nm) is \(1.5 \times 10^9\) M\(^{-1}\) s\(^{-1}\). It has been observed that the absorbance at 460 nm increases with increasing the concentration of \textbf{1}, a similar phenomenon as in the case of the reaction of \(\cdot\text{OH}\).
Reactions of Cl$_2^*$ (at pH 1) and N$_3^*$ (at pH 7) have also produced transient absorption spectrum with 460 nm (see Figure 5.20) and the spectral shapes are identical with that presented in Figure 5.15. Indeed, the reactivity of 1 towards N$_3^*$ ($E^0 = 1.33$ V)$^{61}$ suggests a lower reduction potential of 1. The bimolecular rate constant for the reaction of 1 with Cl$_2^*$ determined from the linear dependence of the pseudo-first-order growth rate constant on the 1 concentration at the absorption maximum of Cl$_2^*$ ($\lambda_{\text{max}} = 360$ nm). The bimolecular rate constant for the Cl$_2^*$ reaction is determined as $2.7 \times 10^9$ M$^{-1}$ s$^{-1}$. The above experimental results for the reactions of 1 with specific one electron oxidants Br$_2^*$, Cl$_2^*$ and N$_3^*$ point out the formation of identical transient species, moreover the time resolved spectra in all these reactions exhibited only one $\lambda_{\text{max}}$ at 460 nm.
Chapter 5

The identity of the transient at 460 nm indicates the formation of 2, however an exhaustive theoretical treatment of *OH reaction with 1 has been carried out which will allow the identification of precise intermediate species. Theoretical calculations unveil the reactive sites in 1, energetics of various reaction possibilities and electronic transition energies of probable transients.

Theoretical study

The BHandHLYP/6-311+G(d,p) level optimized geometry of 1 in solution phase is shown in Figure 5.21a with selected bond length parameters. We got a planar geometry and the shortest bond in the ring is the C5C6 bond. According to Fukui’s frontier orbital approximation, interactions happen only between the frontier molecular orbitals of
reacting entities. The HOMO and LUMO of 1 have energies respectively -7.75 eV and -1.15 eV. The corresponding MO energies of \(^\bullet\)OH are -11.15 eV and -2.57 eV. The LUMO\(^\bullet\)OH – HOMO\(^\bullet\)4-thiouracil energy gap is 5.18 eV whereas LUMO\(^\bullet\)4-thiouracil - HOMO\(^\bullet\)OH energy gap is 10 eV. So the LUMO of \(^\bullet\)OH should interact with the HOMO of 1. Depicted in Figure 5.2b is the isocontour plot of the HOMO of 1 in solution phase. Obviously substantial contributors for the HOMO are S10, C5, and C6 atoms and hence the electrophilic additions of \(^\bullet\)OH might occur at these sites.

**Figure 5.2**: (a) Optimized geometry and (b) HOMO of 4-thiouracil at BHandHLYP/6-311+G(d,p) level in solution phase.

The ionization potential (IP) of 1 is 6.14 eV calculated at BHandHLYP/6-311+G(d,p) level in solution phase, in fact lower than the calculated IP (6.39 eV) of 2-thiouracil. The one electron oxidized species 1a whose solution phase optimized geometry is presented in Figure 5.22, the geometrical parameters especially the bond lengths of thiouracil moiety has modified considerably upon oxidation. The Mulliken spin density is found to be localized on the S atom (1.17 a.u.) of 1a.
Chapter 5

The **OH addition reactions at C5 and C6 are associated with slight puckering of the ring at the addition sites; whereas **OH addition at S10 merely keeping the planarity of the ring structure intact. The main emphasize of all these addition reactions are “direct additions” implies that no intervention of any reactant complexes or adduct formations are characterized by zero activation energies. The optimized geometries of the **OH adducts are presented in Figure 5.2 with selected bond lengths. The unpaired electron spin is localized on the C6 atom (0.94 a.u.) of 3a, while the odd spin is dispersed to the C5 (0.72 a.u.) and S10 (0.54 a.u.) atoms of 3b. The spin density of 3c is more dispersed to the ring and the maximum values are found at C4 (0.54 a.u.) and C6 (0.48 a.u.) atoms. 3b represents the most stable among the **OH addition products; 3a and 3c are respectively 11.53 kcal/mol and 18.57 kcal/mol less stable than 

![Figure 5.2: Solution phase optimized geometries of the transients at BHAndHLYP/6-311+G(d,p) level of theory. Bond lengths are in Å units and maximum spin densities (in a.u.) are given in parenthesis.](image)
3b. The optimized structures of the H-abstracted radicals 4a and 4b produced via the H-abstraction reactions from N1 and N3 atoms with concomitant release of water molecules are represented in Figure 5.22. Thermodynamically 4b is stabilized by 5.45 kcal/mol over 4c. The unpaired spin is localized on the exocyclic S10 atom both in the case of 4a and 4b. However, we have identified the involvement of stable reactant complexes (between *OH and abstractable hydrogens of 1) characterized by hydrogen bonds in the H-abstractions and further details are described in the following section.

**H-abstractions**

In contrast to the *OH addition reactions, the H-abstractions are supposed to commence from reactant complexes represented as 1…*OH(a) (for the H7 abstraction) and 1…*OH(b) (for the H9 abstraction). The 1…*OH(a) and 1…*OH(b) are stabilized respectively by 4.64 kcal/mol and 8.59 kcal/mol in comparison to the separate entities 1 and *OH. Stabilizations of the reactant complexes are due to the relatively strong hydrogen bonds; further the 1…*OH(b) is more stable than the 1…*OH(a) by 3.95 kcal/mol. The odd electron spin is fully localized over the oxygen atom of the *OH in 1…*OH(a), whereas the unpaired spin dispersed into the thiouracil moiety also and the spin density was found to be maximum on the oxygen of the *OH and S10 atoms of 1…*OH(b). The transformations of reactant complexes into the product complexes 4a…H2O and 4b…H2O occur through the transition states TS(a) and TS(b). The activation energy barriers for the 4a…H2O and 4b…H2O formations are 12.79 kcal/mol and 2.43 kcal/mol respectively. TS(a) is characterized by spin localization at the S10 atom, whereas in TS(b) the spin density is found to be maximum on the oxygen of the *OH and S10 atoms. The spin density is located on the S10 atom of both 4a…H2O and 4b…H2O product complexes. The optimized
structures of the H-abstraction pathways in solution are depicted in Figure 5.23.

![Diagram of H-abstraction pathways in solution](image)

**Figure 5.23:** Solution phase optimized geometries of H-abstraction pathways at BHandHLYP/6-311+G(d,p) level. Bond lengths are in Å units and maximum spin densities (in a.u.) are given in parenthesis.

The reaction energy profiles (relative with respect to 1 and *OH) in solution phase for the H-abstractions are depicted in Figure 5.24. The 4b...H₂O is more stable than 4a...H₂O by 4.76 kcal/mol. In due course, the product complexes decompose into the H-abstracted radical and a water molecule. Thermodynamically H9 abstraction is more probable than the H7 abstraction. Obviously the H-abstraction reactions proceed with sufficient activation energy while compared to the *OH addition reactions.
Redox reactions of thiouracils.....

Dimer radical cation

The dimer radical cation 2 is characterized by [−S⋮S−] bond; the calculated S-S bond length is 2.807 Å. The optimized geometry of 2 is presented in Figure 5.25 along with its σ and σ* orbital. The unpaired electron spin is equally shared by the two S-atoms (0.65 a.u. each) constituting the 2c-3e bond showing the symmetrical interaction between the two thiouracil units of 2. Furthermore, the bond length parameters are identical for both the thiouracil units of 2 and the BDE of 2 is calculated as 12.39 kcal/mol. The existence of stable [−S⋮S−] bonded 4-thiouracil containing dimer radical anion has been reported by Wenska et.al\textsuperscript{63}, through combined radiation chemical and theoretical studies. The dimer radical anion was reported to have \( \lambda_{\text{max}} \) at 450 nm and has been considered as the transition from its σ and σ* orbital. Moreover, the S-S bond distance was determined as 2.73 Å. Therefore, 2

![Figure 5.24: Relative energy profile of the H-abstractions in aqueous phase calculated at BHandHLYP/6-311+G(d,p) level of theory.](image)
show spectral similarity by its characteristic absorption around 450 nm to the reported dimer radical anion having the [−S− S−] bond63.

![Figure 5.25: (a) Solution phase optimized geometry of 2 with selected bond lengths (identical for both rings) and spin densities (in a. u.) are in parenthesis. (b) σ and (c) σ* orbitals of 2.](image)

**TDDFT calculations**

The theoretical transition energies and oscillator strengths of the “probable transients” are compiled in Table 5.4. The \( \lambda_{\text{max}} \) values calculated for the transients except 2 falls in the middle-UV spectral region. The predicted \( \lambda_{\text{max}} \) of 2 is in the visible region with a substantially high oscillator strength which is comparable to the experimental spectrum for the reactions of 1 with \( \cdot \text{OH}, \cdot \text{Br}_2^-, \cdot \text{Cl}_2^- \) and \( \cdot \text{N}_3^* \) radicals. The observed spectrum is due to the electronic transition from σ to σ* orbital. Therefore, TDDFT calculations substantiate the experimental results obtained for one electron oxidations of concerned radicals in the present study.
We have seen that, the theoretical calculations agree very well with the pulse radiolysis experimental data for the formation of dimer radical cation in 1, it is worth to extend such calculations to other derivatives of 1. We have selected two derivatives of 1 namely, 4-thiothymine (the 5-methyl derivative) and 4-thiouridine regarding their photophysical properties. Qualitative data such as S-S bond lengths, BDEs and the optical transition moments are drawn for the dimers of these compounds at BHandHLYP/6-311+G(d,p) level of theory in solution phase. It has been found that, as in the case of 2, the dimer radical cations of 4-thiothymine (designated as 5) and 4-thiouridine (designated as 6) are characterized by intermolecular [−S···S−] bond with S-S bond lengths 2.759 Å and 2.823 Å respectively. The S-S bond length calculated for 5 is approximately 0.05 Å shorter while it is 0.02 Å longer for 6 than that noted in 2. The BDEs are respectively 7.54 kcal/mol for 5 and 14.72 kcal/mol for 6. Accordingly, the 4-thiouridine

**Table 5.4**: Calculated $\lambda_{\text{max}}$ and oscillator strengths ($f$) for the plausible transients of 4-thiouracil.

<table>
<thead>
<tr>
<th>Transient</th>
<th>$\lambda_{\text{max}}$ (nm)</th>
<th>$f$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>266</td>
<td>0.257</td>
</tr>
<tr>
<td>3a</td>
<td>245</td>
<td>0.234</td>
</tr>
<tr>
<td>3b</td>
<td>183</td>
<td>0.160</td>
</tr>
<tr>
<td>4a</td>
<td>302</td>
<td>0.153</td>
</tr>
<tr>
<td>4b</td>
<td>265</td>
<td>0.236</td>
</tr>
<tr>
<td>2</td>
<td>457</td>
<td>0.180</td>
</tr>
</tbody>
</table>
dimer radical cation is thermodynamically more stable than parent system 2. Obviously, the methyl substitution at C5 (viz. 4-thiothymine) reduces the stability of 2 by merely half. The unpaired spin is equally distributed over the sulphur atoms of both 5 (0.56 a. u.) and 6 (0.66 a. u.). Moreover, identical bond distances are noted for the thiouracil units of both dimer systems. The \( \lambda_{\text{max}} \) values calculated using TDDFT method for 5 and 6 are respectively 565 nm \( (f = 0.348) \) and 438 nm \( (f = 0.584) \). Obviously, these findings will benefit future empirical studies.

5.7 Conclusions

The reaction of 4-thiouracil (1) with \(^{\bullet}\)OH has been found to produce a transient with \( \lambda_{\text{max}} \) 460 nm. The bimolecular rate constant for \(^{\bullet}\)OH reaction with 1 is evaluated as \( 1.7 \times 10^{10} \text{ M}^{-1} \text{ s}^{-1} \) by competition kinetics method and the observed value point out the diffusion controlled nature of the reaction. Analysis of frontier orbitals of \(^{\bullet}\)OH and 1 showed that, the LUMO of \(^{\bullet}\)OH interacts with the HOMO of 1. All plausible reactions of \(^{\bullet}\)OH with 1 have been theoretically modeled. The transient is ascribed as dimer radical cation (2) via the combination of the radical cation of 1 with another neutral molecule. Fairly high \( (1.8 \times 10^{4} \text{ M}^{-1}) \) value of formation constant has been obtained for the reversible combination of radical cation with neutral molecule. The reactions of 1 with other specific one electron oxidants such as \( \text{Br}_2^{\bullet-}, \text{Cl}_2^{\bullet-} \) and \( \text{N}_3^{\bullet-} \) also pointed out 2 formations. 2 is characterized by \([\text{S}::\text{S}^{-}]\) bond with two electrons filling in the \( \sigma \) orbital and the unpaired electron in the \( \sigma^* \) orbital. The unpaired electron spin is evenly distributed over the two S-atoms, showing a symmetrical interaction of constituent thiouracil moieties in 2. TDDFT calculations of 2 showed a reasonable synchronization between predicted and experimental \( \lambda_{\text{max}} \). The observed \( \lambda_{\text{max}} \) is attributed to the excitation of an electron from doubly occupied \( \sigma \) orbital to the singly occupied \( \sigma^* \) orbital. Theoretical studies showed that
more stable dimer radical cation formation is possible in the case of 4-thiouridine system. The C5 methyl substitution of 1 (4-thiothymine) renders a dimer radical cation with stability roughly half of parent 2 molecule.
Part C

Reduction reactions of 2-thiouracil

Abstract

The reactions of $e_{aq}^-$ and $H^+$ with 2-thiouracil (I) has been studied by using the pulse radiolysis technique and B3LYP/6-31+G(d,p) level DFT calculations. The transient absorption spectrums for the $e_{aq}^-$ reaction showed $\lambda_{max}$ at 350 nm at pH 4.5. The bimolecular rate constant for the $e_{aq}^-$ reaction was determined as $1.5 \times 10^{10}$ M$^{-1}$ s$^{-1}$ monitored from the decay of $e_{aq}^-$ at 700 nm. The absorbance at 350 was unchanged at neutral pH. Phosphate buffers have no effect on the reactions of $e_{aq}^-$ with I; spectral features are identical to that without any buffers (quite unusual when compared to uracil). The $H^+$ reaction at pH 1 was characterized by $\lambda_{max}$ at 340 nm. On the basis of theoretical calculations, O-protonated species is considered as the transient in $e_{aq}^-$ reaction while S-hydrogenated species is suggested as the transient in $H^+$ reaction.
Chapter 5

Publications from this section

5.8 Introduction

The reductive NA damage in aqueous medium via the indirect effect is principally by the hydrated electron \( \text{e}_{\text{aq}}^- \). The \( \text{e}_{\text{aq}}^- \) behave as a nucleophile; react rapidly with organic molecules having low lying vacant orbital. It reacts with nucleobases principally at diffusion controlled rates by forming the radical anion and the latter subsequently protonated by water or acid\(^{28}\). The protonation occurs mainly at the exocyclic oxygen atoms of these pyrimidine and purine systems\(^{64-68}\). However, competing with the rapid protonation at the exocyclic oxygen atoms of nucleobases, there is slow protonation at the ring carbon catalyzed by phosphate buffers. For instance, in the reaction of \( \text{e}_{\text{aq}}^- \) with U the protonation of the corresponding radical anion occurs at O10 (cf. numbering scheme of U in Figure 5.1) in the absence of any buffers. The pKa values of the protonated electron adduct of U and T are respectively 7.3 and 7.2 noted by monitoring the changes in absorbance of the electron adducts as a function of pH. However, in the presence of phosphate buffers, protonation occurs at C6 atom with a pKa > 10. It is also reported that, at neutral pH deprotonation at oxygen is about as facile as protonation, however deprotonation at carbon is very much slower\(^{66}\).

Hydrogen atom (H\(^+\)), the conjugate acid of \( \text{e}_{\text{aq}}^- \), is not an important species in neutral or alkaline solution, but it acts as a reductant in acidic solutions with a reduction potential of -2.31V\(^{28}\). Even though H\(^+\) is a reducing radical, it generally undergoes electrophilic addition reactions with C-C double bonds as \( ^*\text{OH} \) does; while the H-abstraction ability is poor while compared to \( ^*\text{OH} \)\(^{28}\). Keeping the foregoing facts in view, we have studied the reactions of \( \text{e}_{\text{aq}}^- \) and H\(^+\) with 2-thiouracil (1) in aqueous solutions by using pulse radiolysis experiments and addressed by means of B3LYP/6-31+G(d,p) DFT calculations.
5.9 Results and discussions

Hydrated electron reaction

Presented in Figure 5.26 is the transient absorption spectrum ($\lambda_{\max}$ 350 nm) for the reaction of $e_{aq}^-$ with 1 at pH 4.5. The time resolved spectra (not shown here) didn’t exhibit any change in $\lambda_{\max}$ apart from lower $G \times \varepsilon$ values. The $\lambda_{\max}$ reported$^{65}$ for the reaction of $e_{aq}^-$ with U was 310 nm at pH 3.9 and 8. The bimolecular rate constant ($k_2$) for $e_{aq}^-$ reaction with 1 was determined from the slope of linear plots of observed rate constants ($k_{obs}$) against [2-thiouracil] monitored from the decay of $e_{aq}^-$ at 700 nm. The $k_2$ value was determined as $1.5 \times 10^{10}$ M$^{-1}$ s$^{-1}$; designated to the diffusion controlled rate of $e_{aq}^-$ reaction with nucleobases.$^{28}$

Figure 5.26: Transient absorption spectrum for $e_{aq}^-$ reaction with 2-thiouracil at pH 4.5. Spectrum recorded after pulse irradiation of an N$_2$-saturated aqueous solution of 2-thiouracil (1mM) containing 0.3M t-BuOH at (■) 2 µs after the pulse (Dose = 18 Gy/pulse). Inset: (i) Decay kinetics of $e_{aq}^-$ at 700 nm.
The quantification of reducing radicals formed in the reaction of \( e_{aq}^- \) with 1 has been carried out by redox titration method using the reductant MV\(^{2+} \). The \( G(MV^{**}) \) value was calculated as \( 2.28 \times 10^{-7} \ \text{M J}^{-1} \) by probing the formation of MV\(^+ \) at 605 nm. The \( k_{obs} \) for the reaction of the transient (formed from \( e_{aq}^- \) reaction of 1) with MV\(^{2+} \) (5 \times 10\(^{-5} \) M) was obtained as \( 2.17 \times 10^5 \ \text{s}^{-1} \). The transient absorption spectrum for the \( e_{aq}^- \) reaction with 1 at pH 7 is also characterized by \( \lambda_{max} \) 350 nm (see Figure 5.27). However, the spectrum is characterized by higher \( G \times \varepsilon \) values in comparison to the spectrum obtained at pH 4.5. The \( G(MV^{**}) \) was determined as \( 2.71 \times 10^{-7} \ \text{M J}^{-1} \). It was reported that phosphate buffers catalyze the reaction of \( e_{aq}^- \) with U\(^{65} \) and the transient spectrum was characterized with \( \lambda_{max} \) 400 nm. We have studied the effect of phosphate buffer on the reaction of \( e_{aq}^- \) with 1 and the observed spectrum using 0.5 M phosphate buffer (at pH 6) is presented in Figure 5.27.

**Figure 5.27:** Transient absorption spectrum recorded after pulse irradiation of an \( \text{N}_2 \)-saturated aqueous solutions of 2-thiouracil (1mM) containing 0.3M \( t \)-BuOH at (■) 2 \( \mu \)s after the pulse at pH 7 and (▲) in presence of 0.5 M phosphate buffer at pH 6 (Dose = 11.5 Gy/pulse).
It can be seen from Figure 5.2 that, phosphate catalyzed spectrum also characterized by $\lambda_{\text{max}}$ at 350 nm; comparable to the spectrum obtained (at pH 4.5 and 7) without any buffers. The $G(\text{MV}^*)$ was determined as $2.82 \times 10^{-7}$ M J$^{-1}$ and $k_{\text{obs}}$ for the reaction of the transient with MV$^{2+}$ ($5 \times 10^{-5}$ M) was $4.43 \times 10^5$ s$^{-1}$.

**Hydrogen atom reaction**

The transient absorption spectrum obtained for the reaction of H$^*$ with 1 characterized by a $\lambda_{\text{max}}$ at 340 nm is depicted in Figure 5.28. Further, the time resolved spectra doesn’t show any change in $\lambda_{\text{max}}$ and shape except a decrease in $G \times \varepsilon$ values at higher time scales.

![Figure 5.28](image)

**Figure 5.28**: Transient absorption spectrum recorded after pulse irradiation of an N$_2$-saturated aqueous solution of 2-thiouracil (1mM) containing 0.3M t-BuOH at (●) 2 $\mu$s after the pulse at pH 1 (Dose = 21 Gy/pulse). Inset: (i) Formation of MV$^{**}$ at 605 nm (Dose = 6.2 Gy/pulse).

The bimolecular rate constant for the reaction of H$^*$ with 1 was determined at $\lambda_{\text{max}}$ 340 nm of the transient and the calculated value is $5.2 \times 10^9$ M$^{-1}$ s$^{-1}$. The yield of reducing radical produced during the reaction of H$^*$ with 1 is determined by the reaction of MV$^{2+}$ and the
Redox reactions of thiouracils…..

transient; the $G(MV^{*+})$ value was determined as $3.28 \times 10^{-7}$ M J$^{-1}$. The mechanism of the reaction of $e_{aq}^-$ and $H^*$ with 1 is detailed in the following section and important results of B3LYP/6-31+G(d,p) level theoretical calculations in aqueous phase (using PCM solvation model) are also presented.

**Mechanism of $e_{aq}^-$ and $H^*$ reaction**

Typically, $e_{aq}^-$ reaction proceeds by the formation of radical anion as in the case of pyrimidine systems followed by rapid protonation of latter by acids or by water$^{28}$. One should expect greater probability for electron addition at sulphur since the electron affinity of sulphur is more than that of oxygen. The Merz-Sing-Kollman (MK) charges$^{69,70}$ noted at B3LYP/6-31+G(d,p) level in aqueous phase on the radical anion ($1^-\cdot$) of 2-thiouracil are found more at N3, C5, S8 and O10 atoms (Table 5.5).

**Table 5.5: MK charges (in a.u., with hydrogen atom summed to heavy atom) on the radical anions of uracil and 2-thiouracil**

<table>
<thead>
<tr>
<th>Atom</th>
<th>uracil</th>
<th>2-thiouracil</th>
</tr>
</thead>
<tbody>
<tr>
<td>N3</td>
<td>-0.21</td>
<td>-0.19</td>
</tr>
<tr>
<td>C5</td>
<td>-0.26</td>
<td>-0.37</td>
</tr>
<tr>
<td>C6</td>
<td>-0.25</td>
<td>-0.08</td>
</tr>
<tr>
<td>O8(S8)</td>
<td>-0.82</td>
<td>-0.77</td>
</tr>
<tr>
<td>O10</td>
<td>-0.90</td>
<td>-0.89</td>
</tr>
</tbody>
</table>

The highest MK charge is noted for the O10 of $1^-\cdot$ and therefore the protonation should occur at O10. The highest MK charge is noted for the O10 of U radical anion which is in accordance with its protonation predicted by earlier pulse radiolysis experiments$^{28,65}$. Therefore, the
S-substitution doesn’t make any change in the localization of the electron charge in the radical cation of uracil. The schematic of the $e_{aq}^-$ reaction is depicted in Figure 5.28.

The radicals formed by the protonation of $1^{*+}$ at N3, C5, S8 and O10 atoms are represented as 1a, 1b, 1c, and 1d respectively (see Figure 5.29). The B3LYP/6-31+G(d,p) level calculations in solution phase showed that, thermodynamically the C5 protonated 1b is the most stable one. The radicals 1a, 1c, and 1d respectively are 23.86 kcal/mol, 15.7 kcal/mol and 8.7 kcal/mol less stable than 1b. Thus, the kinetic and thermodynamic factors governing the reaction mechanism operating in opposite direction; in the sense that the charge density calculation (MK charges) favors the protonations at O10 atoms more than C5 which results in the thermodynamically most stable species. The intermediate formed in the $e_{aq}^-$ reaction is therefore considered as 1d.

![Figure 5.29: Schematic of the reactions of $e_{aq}^-$ (addition followed by protonation) and $H^+$ with 2-thiouracil.](image)

The characteristic reaction of $H^+$ with pyrimidine bases are addition to electron rich C5 atom$^{28}$. Recall from the HOMO picture...
depicted in Figure 5.3b that, S8 constitute the most electron rich center in 1. Accordingly, one could expect the H⁺ additions at S8 because of its higher contribution towards the HOMO. Therefore the transient formed in the pulse radiolysis experiment can be considered as the S-hydrogenated species 1c.

5.10 Conclusions

We have seen that, the eaq⁻ and H⁺ reactions with 2-thiouracil occur via diffusion controlled rates. The quantification of reducing radicals produced in both reactions was carried out by redox titrations using MV²⁺. The O-protonated species is evolved as the transient system in eaq⁻ reaction, which is characterized by experimental absorption maximum at 350 nm. Also we have seen that, phosphate buffers have no effect on the eaq⁻ reaction and this observation is in complete contradiction to uracil. The electrophilic addition of H⁺ (to the nucleophilic center S8) is conceived as the pertinent reaction comparable to uracil where H⁺ addition occurs at the electron rich C5 atom.
Chapter 5

References


