Oxidation of pyrimidine and purine base components of nucleic acids by hexavalent manganese in aqueous alkaline medium: A kinetic and mechanistic study

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Oxidation of pyrimidine bases viz, uracil, thymine and cytosine and purine bases viz., adenine and guanine by MnO$_4^-$ in aqueous alkali shows first order dependence on [MnO$_4^-$] and fractional order dependence on [nucleic acid base]. The effect of ionic strength is negligible on the rate of oxidation. 5,6-Dihydroxylated compounds have been found to be the products of oxidation in the case of pyrimidines while purines yield the 5-formamidopyrimidines. A mechanism is proposed involving formation of a 5-membered cyclic complex between the substrate and MnO$_4^-$ in a fast step which subsequently disproportionates to give the final products. The formation constants of the intermediate complex and the rates of its disproportionation have been calculated from the derived rate law. The rate of oxidation of pyrimidines is in the order: thymine$>$ uracil$>$ cytosine while in the case of purines the order is guanine$>$ adenine.

Loss of biological activity in living cells caused by ionising radiation is believed to be the result of damage to DNA$^1$. The OH radical produced by ionising radiation react base or sugar moiety of DNA$^2$. The base radicals which are generated by the reaction of OH radicals in DNA might abstract hydrogen from the sugar resulting in the transfer of radical site to the sugar$^3$. The sugar radicals thus produced cause either base release or stand breaks or both in DNA which are severe type of damages to DNA in living cells.

Oxidants like OsO$_4$ have been found to react predominantly with the thymine moiety of $\phi X$-174 single stranded DNA leading to hydroxylation of thymine moiety without any significant strand breaks. The loss of biological activity in $\phi X$-174 DNA was attributed to the hydroxylation of thymine in DNA$^4$. Burton and Riley$^5$ and Beer et al$^6$ studied the reaction of mono- and oligonucleotides with OsO$_4$ and observed that thymine moiety readily reacts with OsO$_4$ while cytosine moiety is less reactive. The thymine moieties in mononucleotides, namely, adenine and guanine are found to be resistant to oxidation by OsO$_4$. It was also reported that OsO$_4$ reacts predominantly with the thymine moiety of denatured DNA, but does not react with the same in native DNA. Recently, there has been significant effort directed towards the selective oxidation of DNA by metal complexes$^7$. Significant progress has been made in achieving selectivity by altering the binding specificity of the complex by appropriate tailoring of the ligands, leading to shape-selective cleavage$^{8,9}$. Efforts have also been directed towards achieving selectivity by altering the reactivity of the metal complex$^{10,11}$. MnO$_4^-$ is reported$^{12,13}$ to selectively degrade thymine residues in DNA. Extensive degradation results in oxidation of cytosine residues also but the purine bases are not affected under mild conditions. Hexavalent manganese oxidations are milder compared to those of heptavalent manganese under otherwise similar conditions$^{14,16}$. Manganate ion, being similar to permanganate in structure, is reported to react with organic compounds containing double bonds in a similar fashion$^{14}$. In fact we have reported the oxidation of uracil and thymine by Mn(VI)$^{10}$ and the rates of oxidation were found to be lower compared to those with permanganate. In an attempt to understand the effects of Mn(VI) on nucleic acids, here we report the kinetics of oxidation of cytosine, adenine and guanine by barium manganate in alkaline medium. For comparison we have incorporated the data on the oxidation of uracil and thymine also.
Materials and Methods

Barium manganate and its stock solutions were prepared as per procedure reported earlier. The solution of BaMnO₄ is stable and homogeneous in 1.0 mol dm⁻³ KOH and the solutions were always prepared fresh just before use. Uracil, thymine, cytosine, adenine and guanine were of Sigma make and used as received.

In the kinetic runs, the reaction was quenched at different times by adding 5 cm³ aliquots to a mixture containing 10 cm³ of 1.0 mol dm⁻³ acetic acid and 10.0 cm³ of 5% KI solution. The unreacted Mn(VI) was then estimated by titrating the liberated I₂ against standard Na₂S₂O₃ solution. The results obtained by this method of analysis were found to agree well with those obtained by spectrophotometric method, where the unreacted Mn(VI) was estimated at regular intervals of time by measuring the absorbance at 600 nm e = 2000 dm³ mol⁻¹ cm⁻¹. The spectrophotometric measurements were carried out on a Hitachi U-1000 spectrophotometer. The HPLC system used for analysis of the products includes Shimadzu LC 6 AD equipment with a dual piston-pump system, a solvent programmer and universal loop injector. Prepacked octadecylsil silica gel ODS Nucleosil semi preparative column (25 cm x 0.46 cm, mean particle size 5 µm) was used. The column effluents were monitored at 220 nm, 260 nm or 280 nm using variable wavelength detector equipped with a 8 µl flow cell. The chromatographic runs were recorded on a Shimadzu model 221-25412 recorder at a chart speed of 5 cm/min.

The chart used in the gradient elution experiments consisted of 50 mM KH₂PO₄ aqueous solution adjusted to pH 5.6 with a pH meter. Before use the phosphate buffer was filtered through a millipore type HA 0.45 µm membrane filter. All mobile phases were degassed using a vacuum pump. The solvent flow rate was kept constant at 0.5 ml/min. The pressure of the pump was maintained at 30 bar. All the HPLC runs were carried out at ambient temperature.

Pyrimidines, viz. uracil, thymine and cytosine, and purines, viz. adenine and guanine (1 x 10⁻³ mol dm⁻³) were allowed to react with MnO₄⁻ in alkaline medium under kinetic conditions. When the green colour of the solution disappeared, the reaction mixture was filtered through a Whatman No. 40 filter paper to remove MnO₂. The reaction mixture after filtration was neutralised with HCl and the pH was brought to 6.0. This solution was then injected into the HPLC system.

The unreacted uracil, thymine and cytosine were detected at their λMAX 260 nm with different retention times while their products were detected at 220 nm. The retention times of the products are comparable with those of the authentic samples of the 5,6-dihydroxylated products of uracil, thymine and cytosine.

The unreacted adenine and guanine were detected at 260 nm while their products were detected at 280 nm with different retention times. The eluent collected at 280 nm was tested for the presence of formaldehyde using chromotropic acid and the pH was brought to 6.0. This solution was then injected into the HPLC system.

Results and Discussion

Kinetic experiments were conducted under pseudo-first order conditions with excess of substrates over Mn(VI). Under these conditions the plot of log [MnO₄⁻] versus time was linear (Fig. 1A), indicating first order dependence on
Table 1—Effect of [substrate] on \(k_{\text{obs}}\) in the oxidation of uracil, thymine, cytosine or adenine by hexavalent manganese in aqueous alkaline medium

\[
\begin{array}{cccccc}
\text{[MnO}_4^-\text{]} & \text{[KOH]} & \text{Temp.} & \text{10}^3 k_{\text{obs}} \text{(s}^{-1}\text{)} \\
7.2 \times 10^{-4} \text{ mol dm}^{-3} & 1.0 \text{ mol dm}^{-3} & 323 \text{ K} & & \\
10^3 [\text{Substrate}] \text{ mol dm}^{-3} & & & & \\
2.0 & 5.13 & 11.5 & 1.67 & 3.64 \\
4.0 & 8.93 & 15.4 & 2.62 & 4.79 \\
6.0 & 12.2 & 20.9 & 3.20 & 5.37 \\
8.0 & 14.3 & 24.8 & 3.52 & 5.96 \\
12.0 & 18.9 & 29.0 & 3.20 & 5.37 \\
16.0 & 22.2 & 41.1 & 7.16 \\
\end{array}
\]

Table 2—Effect of \([\text{MnO}_4^-\text{]}\) and \([\text{guanine}]\) on initial rate in the oxidation of guanine by \(\text{MnO}_4^-\) in aqueous alkaline medium

\[
\begin{array}{cccccc}
[\text{MnO}_4^-\text{]} \text{ (mol dm}^{-3}\text{)} & [\text{guanine}] \text{ (mol dm}^{-3}\text{)} & \text{Initial rate} \text{ (mol dm}^{-3} \text{s}^{-1}\text{)} \\
3.60 & 5.00 & 1.82 \\
7.20 & 5.00 & 3.67 \\
10.8 & 5.00 & 5.25 \\
14.4 & 5.00 & 7.08 \\
21.6 & 5.00 & 10.5 \\
7.20 & 1.25 & 1.30 \\
7.20 & 2.50 & 2.32 \\
7.20 & 10.0 & 5.82 \\
7.20 & 15.0 & 7.70 \\
\end{array}
\]

It is generally accepted that \(\text{MnO}_4^-\) in aqueous solutions reacts rapidly with compounds containing \(\text{C} = \text{C}\) to form an intermediate cyclic 5-membered complex, which subsequently decomposes to give products. In the \(\text{MnO}_4^-\) oxidation of nucleic acid bases it has been reported that \(\text{MnO}_4^-\) shows specificity towards thymine and oxidises it to 5,6-dihydroxy-5,6-dihydrothymine and 5-hydroxy-5-methylbarbituric acid as the primary oxidation products, although further oxidation has been reported to occur under vigorous conditions. Hexavalent manganese in the form of \(\text{MnO}_4^-\) in aqueous alkaline solutions has been reported to react with \(\text{C} = \text{C}\) in a similar fashion but reactions are relatively slow. Under the experimental conditions employed in the present study the negligible effect of added salts on the rate of oxidation indicates the reaction to be of either ion-dipole or dipole-dipole type. \(\text{MnO}_4^-\) in aqueous alkaline solution exists as \(\text{MnO}_6^{2-}\) and therefore the reaction is expected to be between \(\text{MnO}_6^{2-}\) and neutral pyrimidine molecule. Reactions of \(\text{MnO}_6^{2-}\) with organic substrates have been reported to involve a direct two electron transfer and also one electron transfer mediated through \(\text{MnO}_6^{2-}\) (ref. 24). However, we have observed in our systems \(\text{MnO}_6^{2-}\) acting as two electron transfer reagent<sup>14,15</sup>. In the present work also, we have
Table 3—Disproportionation constant (k) and formation constant of the complex (K) and the activation parameters for the oxidation of pyrimidine and purine bases by MnO$_{4}^{-}$ in aqueous alkaline medium at 323 K

<table>
<thead>
<tr>
<th>(a) Pyrimidine bases:</th>
<th>$10^4 \times k$</th>
<th>$K$</th>
<th>$\Delta H^*$</th>
<th>$\Delta S^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>uracil</td>
<td>4.00</td>
<td>72.2</td>
<td>50.0 ± 1.36</td>
<td>-139.9 ± 4.20</td>
</tr>
<tr>
<td>thymine</td>
<td>6.67</td>
<td>78.4</td>
<td>32.6 ± 2.93</td>
<td>-205.2 ± 9.10</td>
</tr>
<tr>
<td>cytosine</td>
<td>0.714</td>
<td>145</td>
<td>27.2 ± 11.2</td>
<td>-239.7 ± 34.6</td>
</tr>
</tbody>
</table>

| (b) Purine bases     |                 |     |             |             |
| adenine              | 0.779           | 428 | 14.3 ± 0.60 | -279.9 ± 1.94 |
| guanine              | 2.22            | 1500| 32.3 ± 0.06 | -215.7 ± 0.80 |

Error limits are standard deviations.

checked the possible involvement of MnO$_{4}^{-}$ in the reaction. MnO$_{4}^{-}$ has been reported to have strong absorption at 700 nm$^{14}$, but we have not observed any absorption at 700 nm with the reaction mixture and also there is no polymerisation with added acrylamide suggesting that neither MnO$_{4}^{-}$ nor any radicals are involved in the reaction, both with pyrimidines and purines. The fractional order dependence of reaction rate on [pyrimidine] indicates that it may be involved in complex formation with MnO$_{4}^{-}$. In fact in the oxidation of uracil by permanganate in alkaline medium formation of cyclic five membered diester type stable complex was observed using stopped flow technique$^{12}$. Therefore, it is reasonable to assume the formation of such a cyclic intermediate complex with MnO$_{4}^{-}$ as well. In view of the experimental results obtained and the above discussion, the reaction Scheme 1 has been proposed for MnO$_{4}^{-}$ oxidation of pyrimidines.

Beer et al$^{6}$ have studied the oxidation of purine mononucleotides by OsO$_{4}$ and reported that the purine bases are not affected by OsO$_{4}$. Similar observations were also made by Iida and Hayatsu$^{21}$ with permanganate. Jones and Walker$^{22}$ reported degradation of guanine moiety in DNA by permanganate under vigorous conditions. However, no attempt has been made on the kinetic and mechanistic aspects of oxidation of purines by metal ions. Under the experimental conditions employed the negligible effect of added salts on the rate of oxidation of purines by MnO$_{4}^{-}$ indicates the reaction to be of ion-dipole type. The fractional order dependence of rate on [purine] indicates that it may be involved in complex formation with MnO$_{4}^{-}$. The metal ion interaction studies carried out by Chatterji and Nandi$^{23}$ with purines suggest that N(7) in purines is a potential metal binding site. The calculated charge density and localisation energy values of electrophilic attack at various carbon atoms in purine ring suggest that C(8) is more favourable for electrophilic attack$^{28}$. From the kinetic results, product analysis and the above discussion we propose that the MnO$_{4}^{-}$ attacks N(7)....C(8) double bond leading to the formation of a cyclic intermediate as shown in the Scheme 2,

![Scheme 2](image_url)

From the Schemes 1 and 2, similar rate laws can be derived for the oxidation of pyrimidines and purines by MnO$_{4}^{-}$. The rate law derived is given by Eq. (1).

$$- \frac{d[MnO_{4}^{-}]}{dt} = k \frac{[substrate] \cdot [MnO_{4}^{-}]}{1 + [substrate]} \quad (1)$$

$$- 2.303 \frac{d\log[MnO_{4}^{-}]}{dt} = k_{obs} \frac{Kk[substrate]}{1 + K[substrate]} \quad (2)$$

It is to be pointed that a generalised law with a summation of terms in the denominator may indicate either a change in the rate limiting step or that the substrate is split substantially between two forms in a prior equilibrium. A change in the rate limiting step would lead to changes in the rate law as the concentrations of the reactants are varied. But, in our study, the rate law (Eq. 1) was found to be applicable over the entire range of concentrations used, indicating no change in the rate limiting step. The other possibility that the substrate is split substantially between two forms in a prior equilibrium is also not applicable in our system because of the obvious reasons that the equilibrium involves the substrate and the reagent and not the substrate and an acid or base. The rate law explains well the first order dependence of rate on [Mn(VI)] and fractional order dependence on [substrate]. Taking the reciprocal of Eq. (2), we get

$$\frac{1}{k_{obs}} = \frac{1}{1 + Kk[substrate]} + \frac{1}{k} \quad (3)$$
According to Eq. (3) the plot of 1/$k_{obs}$ versus 1/
[substrate] should be linear and was found to be so for both pyrimidines and purines [Figs 2(A) & (B)], supporting the proposed mechanism. The formation constant of the intermediate cyclic complex (K) and the disproportionation constant of the complex (k) have been calculated from the intercept and slopes of these plots (Table 3).

The reactivity of pyrimidines is found to be: thymine > uracil > cytosine. The high reactivity of thymine could be due to the (+I) inductive effect of the methyl group facilitating the formation of the cyclic intermediate, which is evident from the formation constant values (Table 3). Similar observations were also made by Freeman et al. concerning the oxidation of pyrimidines by potassium permanganate. The data in Table 3 show that guanine reacts faster than adenine.

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References

5 Burton K & Riley W T, Biochem J, 98 (1966) 70.