Research Article

Kinetic and Mechanistic Study of Oxidation of Pyrimidines by Sodium Chromate and Hydrogen Peroxide System in Presence of Ascorbic acid and Protection by Riboflavin

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Abstract

The oxidation of pyrimidines viz., thymine and uracil with sodium chromate and hydrogen peroxide (Cr(VI) - H_2O_2) system in presence of ascorbic acid at pH 8 has been carried in tris HCl buffer. The reactions are followed by measuring the absorbance of pyrimidine bases at their respective λ_{max}. The rates of oxidation of pyrimidines increased with increase in [chromate], [hydrogen peroxide] and [ascorbic acid]. The plot of log (initial rate) versus log [chromate], and log (initial rate) versus log [ascorbic acid] was found to be linear with slope less than one indicating fractional order dependence on [chromate] and [ascorbic acid]. The fractional order dependence of reaction rate on [ascorbic acid] and [chromate] indicates that reaction might be proceeding through the initial formation of a complex between ascorbic acid and Cr (VI). The first order dependence of rate of oxidation on [H_2O_2] suggests that Chromium-Ascorbate complex might react with H_2O_2 in the rate determining step via Fenton like reaction to generate OH radicals. An increase in [pyrimidine] at constant [chromate], [hydrogen peroxide] and [ascorbic acid] has no significant increase of rate of oxidation suggests that OH radicals react with pyrimidines in a fast step to form product. It has been found that riboflavin protects thymine and uracil to the extent of 71.42 % and.70.77% at 128µM concentration of riboflavin from OH radicals.

Keywords: Oxidation of pyrimidines, Chromium (VI), Hydrogen peroxide, ascorbic acid, Chromium-Ascorbate complex, Protection by riboflavin

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Introduction

Chromate, Cr(VI), as a human carcinogen has been established, although its mechanism of action has not been clearly defined [1, 2]. The reaction of DNA with chromate causes a number of putative lesions in cellular systems including oxidized nucleic acid bases, abasic sites, DNA strand breaks, inter- and intrastrand cross-linked adducts and DNA–protein cross-links [3-7]. The tetrahedral anionic conformation of Cr(VI) facilitates its active transport into cell systems through the phosphate and sulfate cellular transport systems [8].

However, it is reported that Cr(VI) is not the oxidation state that reacts with isolated DNA, reduction of Cr(VI) by cellular reductants has been thought to be an important step in the mechanism of Cr(VI)-induced DNA damage [8, 9]. Cr (V) species has been reported to be generated in the reduction of Cr(VI) by various biological systems [8], in particular microsomes [10, 11], mitochondria [12], superoxide radical [13], certain flavoenzymes [14-16], mitochondrial electron transfer chain complexes [17], ascorbate [18,19] and thiol- and diol-containing molecules [8, 20-22]. Regarding the mechanism of Cr (VI) Carcinogenicity, Jennette [10] postulated that Cr (V) is the ultimate carcinogenic form of chromium compounds.

The first evidence for the role of $Cr^{V}(O_2)_4^{3-}$ in the Cr (VI) - mediated OH radical generation was reported by Kawanishi et.al [23]. They observed ($Cr^{V}(O_2)_4^{3-}$) formation by E.S.R from a mixture containing 40mM Na_2CrO_4 and 400 mM H_2O_2 at pH 8. However, the concentrations used for both Cr (VI) and H_2O_2 were orders of magnitude higher than in any vivo estimate. Later Aiyar et al [24] reaffirmed the Kawanishi model of $^{\cdot}OH$ radical generation from a mixture of Cr (VI) and H_2O_2 i,e tetraperoxochromium (V) complex ($Cr^{V}(O_2)_4^{3-}$) was the species responsible for OH radical generation.

Later shi et al [25] studied using ESR and spin trapping techniques and found that ($Cr^{V}(O_2)_4^{3-}$) is not formed in any significant quantity in the reaction of chromate with biologically relevant reductants such as glutathione,

NADPH, Ascorbate, Vitamin B_2. Decomposition of $Cr^{V}(O_2)_4^{3-}$ or its reaction with H_2O_2 does not generate any significant amount of $^{\cdot}OH$ radicals. The major Cr (V) Species formed are complexes of Cr (V) with reductant moieties as ligands. These Cr(V) complexes generate $^{\cdot}OH$ radicals from H_2O_2 via Fenton like reaction.

Treatment of the cells with vitamin B2 (Riboflavin) prior to chromate treatment led to an increase in DNA single strand breaks over that observed upon treatment with chromate alone [26], presumably due to an increase in chromium (V)-related hydroxyl radical production. On the other hand, incubation of the V-79 cells with vitamin E, a hydroxyl radical scavenger, prior to chromate treatment led to a decrease in DNA single-strand breaks [27].

In animal studies, chromium (VI)-induced DNA damage has been found to be tissue-dependent. DNA - interstrand crosslinks and DNA-protein crosslinks were known in the livers of chick embryos treated with chromium (VI), but in the red blood cells, DNA damage was observed primarily in the form of strand breaks [28]. This may reflect the fact that reduction of chromium (VI) occurs by different metabolic pathways in the various tissues.

One way to obtain information about these species is by studying the products of the reaction of chromate with DNA, or individual nucleotides, nucleosides, nucleic acid bases to get an insight into the mechanism of oxidation of DNA constituents. In the present study an attempt has been made to investigate the mechanism of damage to DNA constituents by OH radicals produced from chromium (VI) with hydrogen peroxide (Cr(VI) - H_2O_2) system in presence and absence of naturally available antioxidants.

Experimental

Thymine, uracil, sodium chromate, riboflavin and ascorbic acid were from sigma and used as such. The solutions were prepared afresh with double distilled water and standardized. The Emsure® Hydrogen Peroxide 30% was used for analysis and standardized using the method described in the literature [29]. The concentration of pyrimidines was determined by measuring the absorbance at their respective λ_{max} from the known absorption coefficient values. In a typical reaction required amounts of pyrimidines viz., thymine/ uracil and H_2O_2 solution were then injected as aqueous solution into the mixture of ascorbic acid and sodium chromate solutions present in a 1-cm path length quartz cuvette suitable for absorbance measurements. The progress of the reactions were followed by measuring the absorbance at λ_{max} of pyrimidines on double beam UV-visible Spectrophotometer model T60U of Lab India at different time intervals from which rate of oxidation of pyrimidines were calculated.

The HPLC system used for analysis of products includes schimadzu LC-10AT equipment with dual piston pump system, a solvent programmer and Reodhyne injector model 7725 fitted with 20µl loop. A prepacked octadecylsilyl silica gel ODS hypersil column 25cm × 0.46cm, mean particle size 5µm was used. The column effluents were monitored at 280nm, using variable wavelength SPD-10A UV-Visible detector equipped with 8 µl flow cell and attached to C-R7Ae plus chromatographic integrator. Samples were eluted with aqueous solutions containing 5% (v/v) methanol and buffered with 10mM KH_2PO_4 solution adjusted to pH 8. Before use the phosphate buffer was filtered through Millipore type HA 0.45 µm membrane filter. All mobile phases were degassed using a vaccum pump. The solvent flow rate was kept at 0.5ml/min and all the HPLC runs were carried out at ambient temperatures.

Results and discussion.

The oxidation of pyrimidines viz., thymine and uracil with sodium chromate and hydrogen peroxide (Cr(VI) - H_2O_2) system in presence and absence of ascorbic acid at pH 8 has been carried in tris HCl buffer. Pyrimidines were not oxidized even with highest concentration of sodium chromate (1×10^{-4} M) and hydrogen peroxide (5×10^{-3}M) system in the absence of ascorbic acid but in presence of ascorbic acid ($1x10^{-5}$M), pyrimidines were oxidized even with concentration as low as of sodium chromate (1×10^{-8} M) and hydrogen peroxide (5×10^{-3} M). The oxidation of pyrimidine bases with Cr(VI) - H_2O_2 system in presence of ascorbic acid were carried out at different [chromate] varying from 0.25×10^{-4} to 2×10^{-4} M keeping [ascorbic acid], [pyrimidine] and [H_2O_2] constant. The plot of log (initial rate) versus log [chromate] was found to be linear with slope less than one indicating fractional order dependence on chromate (**Figure 1**). The effect of [ascorbic acid] varying between $1 \times 10^{-5} – 4 \times 10^{-5}$ M was studied keeping [H_2O_2], [pyrimidine] and [chromate] constant. The plot of log (initial rate) versus log [ascorbic acid] was found to be linear with slope less than one indicating fractional order dependence on ascorbic acid (**Figure 2**).

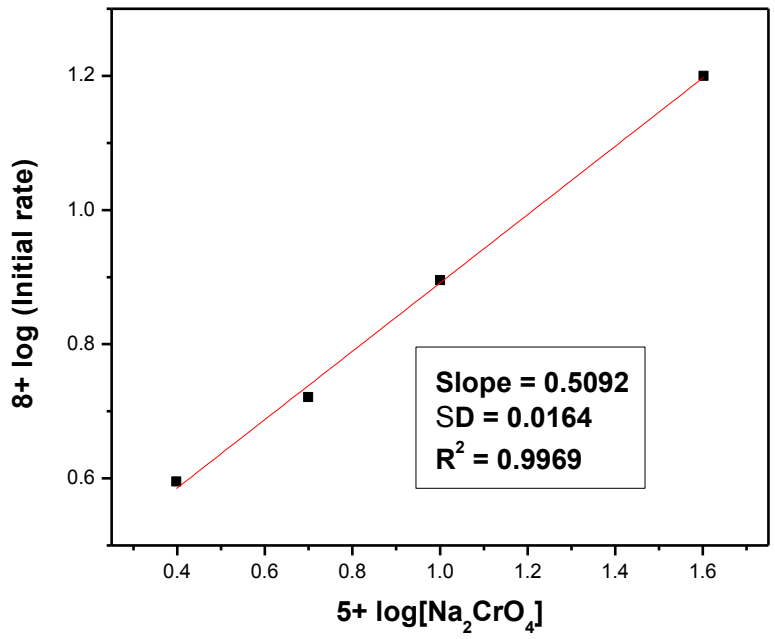


Figure 1 Order in [Chromate] in the oxidation of uracil with Cr(VI)- H_2O_2 system in presence of ascorbic acid

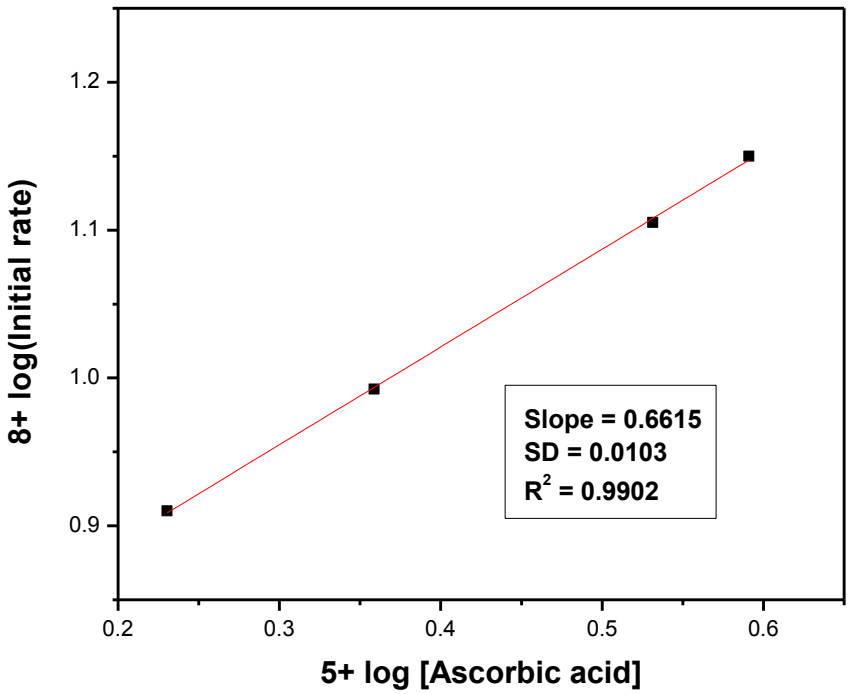


Figure 2 Order in [Ascorbic acid] in the oxidation of uracil with Cr(VI)- H2O2 system in presence of ascorbic acid

An increase in [H_2O_2] was found to increase the rate of oxidation and order with respect to [H_2O_2] has been found to be unity from the slopes of log (initial rate) versus log [H_2O_2]. The concentration of H_2O_2 was varied between 1×10^{-3}- 1×10^{-2} M. An increase in [pyrimidine] at constant [chromate], [hydrogen peroxide] and [ascorbic acid] has no significant effect on rate of oxidation of pyrimidines. The decrease of absorbance of pyrimidine in the reaction mixture at different intervals of time has been obtained by using chromate, hydrogen peroxide and ascorbic acid mixture as reference in double beam spectrophotometer. From these the rates of oxidation of pyrimidines were calculated from the plots of absorbance versus time using microcal origin computer program on personal computer.

Shi. et.al reported that as low as 25mM of $K_2Cr_2O_7$ and 25mM H_2O_2 were required to generate detectable amount of ($Cr^V(O_2)_4^{3-}$) ions at physiological pH (pH = 7.2) [25]. In contrast in presence of certain flavoenzymes such as GSSG-R, even 0.5mM of $K_2Cr_2O_7$ and 1mM NADPH generated intense Cr(V) ESR signal which was assigned to Cr(V)-NADPH complex formation [14-16]. The Cr(V)-complex formation is much more efficient than ($Cr^V(O_2)_4^{3-}$) formation from direct reaction of Cr(VI) and H_2O_2 [25]. Mixture of $K_2Cr_2O_7$ (10mM) and ascorbic acid (10mM) in phosphate buffer (pH = 7.2) generated an E.S.R signal at g = 1.9794 which was assigned to Cr (V)-Ascorbate complex [25, 18, 19]. No ($Cr^V(O_2)_4^{3-}$) ESR Signal was detected. When H_2O_2 and DMPO (as a spin trap) were added, a 1:2:2:1 quartet with hyperfine splitting of $a_N = a_H$ = 14.9G was observed. Based on this splitting [20] the 1:2:2:1 signal was assigned to the DMPO/OH adduct, as evidence of OH radical generation. Upon addition of H_2O_2, however, Cr(V) became non-detectable indicating that OH radicals were generated in the reaction between Cr(V)-ascorbate complex and H_2O_2 via a Fenton-like mechanism[25].

In the present work, the fractional order dependence of reaction rate on [ascorbic acid] and [chromate] indicates that reaction might be proceeding through the initial formation of complex between ascorbic acid and Cr (VI) to give Cr(V)-ascorbate complex. The first order dependence of rate of oxidation of pyrimidine on [H_2O_2] suggests that Cr(V)-Ascorbate complex reacts with H_2O_2 in the rate determining step via Fenton like reaction to generate OH radicals. With the addition of ethanol oxidation of pyrimidines decreased indicating that OH radicals are generated in our system. The rate constant of OH radical with ethanol is reported to be $1.8 \pm 0.2 \times 10^9$ $dm^3mol^{-1}s^{-1}$ [30] and the rate constant of OH radical with thymine and uracil are reported to be $6.3x10^9$ $dm^3mol^{-1}s^{-1}$ and $6.5\ x10^9$ $dm^3mol^{-1}s^{-1}$ respectively [31, 32]. Hence in this system there might be a competition between pyrimidine and ethanol for OH radicals. The relative amounts of OH radicals reacting with pyrimidine decreases with increasing [ethanol]. An increase in [pyrimidine] at constant [chromate], [hydrogen peroxide] and [ascorbic acid] has no effect on rate of oxidation suggests that OH radicals react with pyrimidines in the fast step to form product. It has been reported [33] that OH radicals attack pyrimidines viz., thymine and uracil at C5/C6 double bond leading to C5-OH (6-yl radical) and/or C6-OH (5-yl radical) adduct radicals. The radicals formed by attachment of OH radicals to C6 position of pyrimidine have oxidizing properties with respect to N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD): those produced by addition of OH radicals to C5 position of pyrimidine are able to reduce tetranitro-methane (TNM)[34]. These differences in redox properties have been utilized to determine OH attachment between C5 and C6. The OH radical addition has been found to be mainly at C5 position for 1.3-dimethyluracil and 1,3,6-trimethyluracil while it is at C6 for thymine. This difference in reactivity has been attributed to steric hindrance of methyl group at C5 position in thymine [35-37]. It is also reported that C5-OH adduct radicals are further oxidized to substituted isobarbituric acid as the main product while C6-OH adduct radicals in thymine are oxidized to 5,6-dihydroxythymine[38, 39].

The oxidation product of thymine has been identified as 5,6-dihydroxythymine. The retention times of thymine and 5,6-dihydroxythymine has been found to be 14.35 min and 3.94 min respectively which was further confirmed by the authentic sample. The oxidation products of uracil has been identified as isobarbituric acid and 5,6-dihydroxyuracil. The retention times of uracil, isobarbituric acid and 5,6-dihydroxyuracil has been found to be 6.27 min,5.03 and 3.94 min respectively which was further confirmed by the authentic sample by HPLC with 5% methanol buffered with 10mM phosphate buffer at pH 8 with ODS column at flow rate of 0.5 ml/min. Therefore it is suggested that OH radicals generated in our system attacks pyrimidines at C5/C6 double bond to form product.

Effect of pH

With increase in pH rate of oxidation of pyrimidines have been found to increase with Cr(VI)-H_2O_2 system in presence of ascorbic acid. The Cr(V)-Ascorbate complex at g_{iso} = 1.9791 is much more stable in moderately acidic solution (pH = 3-6) than in neutral and alkaline solutions [40]. A.D. Bokare et al [41] reported that tetraperoxochromate(V) ($Cr^V(O_2)_4^{3-}$) production is reduced with increase in pH in the reaction of Cr(VI) (2mM) and hydrogen peroxide (20mM). The production of ($Cr^V(O_2)_4^{3-}$) is immediate at pH = 3, but was markedly reduced at pH = 6 and negligible at pH = $\geq$ 8. When H_2O_2 is added to chromate solution the absorption peak at 352nm (lambda max of chromium) decreased and absorbance above 500nm increased indicating the formation of ($Cr^V(O_2)_4^{3-}$). The increase in absorbance above 500nm is decreased as pH is increased from pH=3-6 and negligible at pH =8. Increase in rate of oxidation of pyrimidines with pH clearly shows that oxidation occurs by OH radicals generated by reaction of H_2O_2 with Cr(V) - ascorbate complex via Fenton like reaction (**Tables 1** and **2**).

Table 1 Effect of pH on rate of oxidation of thymine with Cr(VI)-H_2O_2 system in presence of ascorbic acid in aqueous solutions. $[H_2O_2] = 5.0 \times 10^{-3}$mol dm^{-3}, [Thymine] = 1.0×10^{-4} mol dm^{-3}, $[Na_2CrO_4] = 1.0 \times 10^{-4}$mol dm^{-3}, [Ascorbic acid] = 1.5×10^{-5} mol dm^{-3}. Temperature = 298K

S.No	pH	10^7 × (Initial rate) (mol dm^{-3} s^{-1})
1	8.0	1.478
2	6.0	1.032
3	4.5	0.809

Anti oxidant activity of Riboflavin

The initial rates of oxidation of pyrimidines by Cr (VI) - H_2O_2 system in presence of ascorbic acid have been found to decrease with increase in [Riboflavin] indicating that riboflavin is protecting the pyrimidines from OH radicals generated with Cr (V)-ascorbate complex reaction with H_2O_2 via Fenton like reaction (**Tables 3** and **4)** and (**Figures 3** and **4)**.

Table 2 Effect of [pH] on rate of oxidation of uracil with Cr(VI)-H_2O_2 system in presence of ascorbic acid in aqueous solution. $[H_2O_2] = 5.0 \times 10^{-3}$ mol dm^{-3}, [Uracil] = 1.0×10^{-4} mol dm^{-3}, $[Na_2CrO_4]$ =1.0×10^{-4} mol dm^{-3}, [Ascorbic acid] = 1.5×10^{-5}moldm^{-3}, Temperature = 298K

S.No	pH	10^7 × (Initial rate) (mol dm^{-3} s^{-1})
1	8.0	1.135
2	6.0	0.621
3	4.5	0.365

Table 3 Effect of [Riboflavin] on rate of oxidation of thymine with Cr(VI)-H_2O_2 system in presence of ascorbic acid in aqueous solution. $[H_2O_2] = 5.0 \times 10^{-3}$mol dm^{-3}, [Thymine] = 1.00×10^{-4}mol dm^{-3}, $[Na_2CrO_4] = 1.0 \times 10^{-4}$mol dm^{-3}, [Ascorbic acid] = 1.5×10^{-5}mol dm^{-3} pH= 8, Temp = 298 K

S.No	10^6 x [Riboflavin] (mol dm^{-3})	10^7 x rate (mol dm^{-3} s^{-1})	p	% Scavenging (1-p)×100)
1	0.00	1.478	-	-
2	8.00	1.270	0.850	13.50
3	16.0	1.124	0.762	23.80
4	24.0	1.000	0.680	30.23
5	32.0	0.911	0.615	38.45
6	48.0	0.771	0.516	48.37
7	64.0	0.665	0.444	55.54
8	128	0.424	0.285	71.42

Table 4 Effect of [Riboflavin] on rate of oxidation of Uracil with Cr(VI)-H_2O_2 system in presence of ascorbic acid in aqueous solution. $[H_2O_2] = 5.0 \times 10^{-3}$ mol dm^{-3}, [Uracil] = 1.00×10^{-4} mol dm^{-3}, $[Na_2CrO_4] = 1.0 \times 10^{-4}$ mol dm^{-3}, [Ascorbic acid] = 1.50×10^{-5}mol dm^{-3} pH= 8, Temp = 298 K

S.No	10^6 x [Riboflavin] (mol dm^{-3})	10^7 x rate (mol dm^{-3} s^{-1})	p	% Scavenging (1-p)×100)
1	0.00	1.135	-	-
2	8.00	0.945	0.868	13.14
3	16.0	0.871	0.767	23.24
4	24.0	0.787	0.687	31.23
5	32.0	0.707	0.622	37.71
6	48.0	0.592	0.524	47.59
7	64.0	0.511	0.452	54.77
8	128	0.334	0.292	70.77

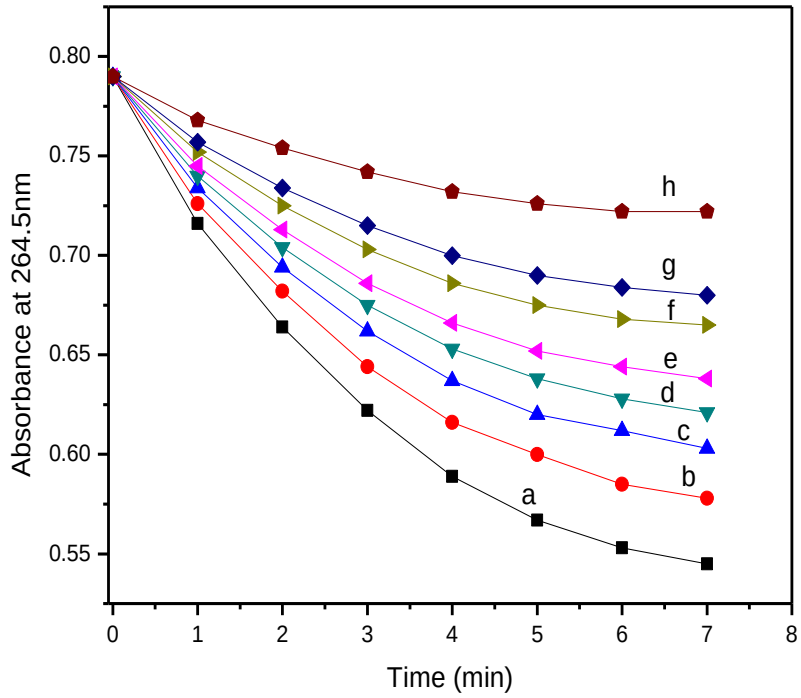


Figure 3 Effect of Riboflavin on the oxidation of thymine with Cr(VI)-H_2O_2 system in presence of ascorbic acid in aqueous solution. $[H_2O_2]$ = 5.0 × 10^{-3}mol dm^{-3}, [Thymine] = 1.00× 10^{-4}mol dm^{-3}, $[Na_2CrO_4]$ = 1.0 × 10^{-4}mol dm^{-3}, [Ascorbic acid] = 1.50 × 10^{-5}mol dm^{-3}, [Riboflavin] = a) 0.00 b) 8.0 ×10^{-6}mol dm^{-3} c) 16.0 ×10^{-6}mol dm^{-3}, d)24.0 ×10^{-6}mol dm^{-3} e) 32.0 ×10^{-6}mol dm^{-3} f) 48.0 ×10^{-6}mol dm^{-3}, g) 64.0 ×10^{-6}mol dm^{-3} h) 128.0 ×10^{-6}mol dm^{-3}

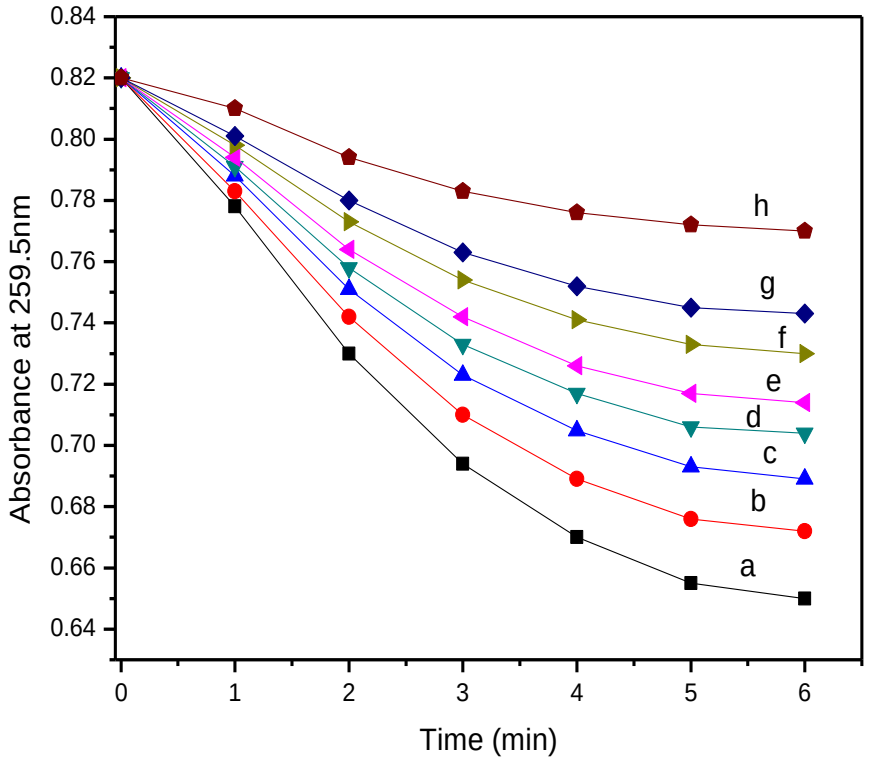


Figure 4 Effect of Riboflavin on the oxidation of uracil with Cr(VI)-H_2O_2 system in presence of ascorbic acid in aqueous solution. $[H_2O_2]$ = 5.0 × 10^{-3} mol dm^{-3}, [uracil] = 1.0× 10^{-4} mol dm^{-3}, $[Na_2CrO_4]$ = 1.0 × 10^{-4} mol dm^{-3}, [Ascorbic acid] = 1.50 × 10^{-5} mol dm^{-3}, [Riboflavin] = a) 0.00 b) 8.0 ×10^{-6} mol dm^{-3} c) 16.0 ×10^{-6} mol dm^{-3}, d) 24.0 ×10^{-6} mol dm^{-3} e) 32.0 ×10^{-6} mol dm^{-3} f) 48.0 ×10^{-6} mol dm^{-3}, g) 64.0 ×10^{-6} mol dm^{-3} h) 128.0 ×10^{-6}mol dm^{-3}

In this system there is a competition between pyrimidine and riboflavin for OH radicals. The relative amounts of OH radicals reacting with pyrimidine decreases with increasing [riboflavin]. The rate constant of riboflavin with hydroxyl radical has been reported to be $1.2 \pm 0.05 \times 10^{10} dm^3 mol^{-1} s^{-1}$ over the pH range 1–11 [42]. The rate constant of hydroxyl radical with ascorbic acid at physiological pH has been reported to be 2.7×10^5 $dm^3 mol^{-1} s^{-1}$ [43]. This indicates that ascorbic acid is not specific hydroxyl radical scavenger at physiological pH. Since the rate constant of OH radical with riboflavin is much higher than rate constant of OH radical with ascorbic acid, it is understood that ascorbic acid does not compete for OH radicals under the experimental conditions.

The rate constant of the reaction of the OH radical with thymine and uracil were reported [30, 31] to be $6.3x10^9$ $dm^3 mol^{-1} s^{-1}$ and $6.5x10^9$ $dm^3 mol^{-1} s^{-1}$ respectively. The rate constant of the reaction of the OH radical with riboflavin has been calculated by the pyrimidine competition method at pH 8 in tris HCl buffer, which is very similar to the one chosen by Akhalaq et al [44] to determine the rate constant for the reaction of OH radicals with polyhydric alcohols in competition with KSCN. Solutions containing pyrimidines with Cr(VI)-H_2O_2 system in presence of ascorbic acid and varying amounts of riboflavin were made to react for 4 minutes and decrease of absorbance of pyrimidines was measured. The decrease of absorbance of pyrimidine reflects the number of OH radicals that have reacted with pyrimidine. From the rate constant of reaction of pyrimidine with OH radicals ($k_{\text{Thymine + OH}} = 6.3x10^9$ $dm^3 mol^{-1} s^{-1}$) and ($k_{\text{Uracil + OH}} = 6.5 \times 10^9$ $dm^3 mol^{-1} s^{-1}$, The rate constant of OH radicals with riboflavin ($k_{\text{Riboflavin + OH}}$) can be calculated using equation (1).

$$\frac{[\text{Absorbance of Pyrimidine}]_0}{[\text{Absorbance of Pyrimidine}]_{\text{Riboflavin}}} = 1 + \frac{k_{(OH + Riboflavin)}[Riboflavin]}{k_{(OH + \text{Pyrimidine})}[\text{Pyrimidine}]} - (1)$$

Where $[\text{Absorbance of pyrimidine}]_0$ and $[\text{Absorbance of pyrimidine}]_{\text{riboflavin}}$ indicate the decrease in the absorbance of pyrimidine in the absence and presence of riboflavin respectively, in the same interval of time. Experiments of this kind should be carried out with great accuracy. The rate constant for the reaction of OH radical with riboflavin has been calculated with seven different concentrations of riboflavin and average value obtained is 1.23 ± 0.12 $x10^{10} dm^3 mol^{-1} s^{-1}$.

The probability of OH radicals reacting with pyrimidines {p (OH + pyrimidine)} is calculated using the following equation.

$$p(\cdot OH + \text{Pyrimidine}) = \frac{[\text{Pyrimidine}]\ k_{\text{pyrimidine}}}{[\text{Pyrimidine}]\ k_{pyrimidine} + [\text{Riboflavin}] k_{\text{Riboflavin}}} - (2)$$

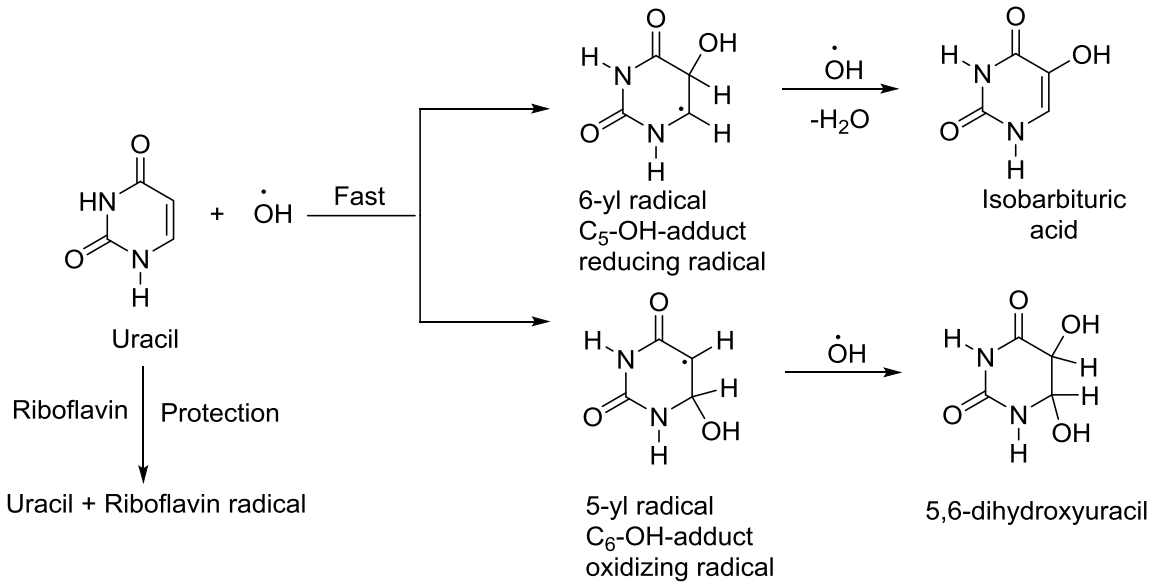
$k_{\text{Pyrimidine}}$ and $k_{\text{riboflavin}}$ are the rate constants of OH radical with pyrimidine and riboflavin respectively. From the rate constant of OH radical with riboflavin and Pyrimidine (Equation (2)), the fraction of OH radicals scavenged by riboflavin (Percentage scavenged = (1 – p) x 100) at different [riboflavin] were calculated (Table.3 &4). These values were a measure of protection of pyrimidine due to scavenging of OH radicals by riboflavin. It has been found that riboflavin is protecting thymine and uracil to the extent of 71.42 % and.70.77% at 128µM concentration of riboflavin from OH radicals.

Based on the above results and discussion the following mechanism has been proposed taking uracil as an example in scheme 1

Scheme 1

$$\text{Cr (VI)} + \text{Ascorbic acid} \rightleftharpoons \text{Cr (V) - (Ascorbate) Complex}$$

$$\text{Cr (V) - (Ascorbate) Complex} + H_2O_2 \xrightarrow{\text{RDS}} \dot{O}H + \bar{O}H + \text{Cr(VI)}$$



Conclusions

The present study exhibit that under biologically relevant conditions, mixture of Cr(VI) and H_2O_2 does not oxidize pyrimidines by OH radicals generated in this system but Cr(VI) Concentration as low as 1×10^{-8} M and H_2O_2 5×10^{-3} M could oxidize pyrimidines in presence of ascorbic acid. Riboflavin is found to protect thymine and uracil oxidation by OH radicals to the extent of 71.42 % and.70.77% at 128µM concentration of riboflavin.

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