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KINETICS AND MECHANISM OF PROTECTION OF THYMIDINE FROM SULPHATE RADICAL ANION BY CAFFEIC ACID UNDER ANOXIC CONDITIONS

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ABSTRACT

The oxidation of thymidine by $SO_4^{\bullet-}$ has been followed by measuring the absorbance of thymidine at 267 nm spectrophotometrically. The rates and the quantum yields (ϕ) of oxidation of thymidine by sulphate radical anion have been determined in the presence of different concentrations of caffeic acid. Increase in [caffeic acid] is found to decrease the rate of oxidation of thymidine suggesting that caffeic acid acts as an efficient scavenger of $SO_4^{\bullet-}$ and protects thymidine from it. Sulphate radical anion competes for thymidine as well as for caffeic acid. The quantum yields of photooxidation of thymidine have been calculated from the rates of oxidation of thymidine and the light intensity absorbed by Peroxydisulphate (PDS) at 254 nm, the wavelength at which PDS is activated to sulphate radical anion. From the results of experimentally determined quantum yields (ϕ_{exptl}) and the quantum yields calculated (ϕ_{cal}) assuming caffeic acid acting only as a scavenger of $SO_4^{\bullet-}$ radicals show that ϕ_{exptl} values are lower than ϕ_{cal} values. The experimentally found quantum yield values at each caffeic acid concentration and corrected for $SO_4^{\bullet-}$ scavenging by caffeic acid (ϕ^1) are also found to be greater than ϕ_{exptl} values. These observations suggest that the thymidine radicals are repaired by caffeic acid in addition to scavenging of sulphate radical anions.

Key words: Oxidation of caffeic acid, Repair of thymidine by caffeic acid, Oxidation by sulphate radical anion.

INTRODUCTION

The lethal effects of ionizing radiation on cellular systems involve radical induced chemical changes in essential biomolecules, particularly in deoxyribonucleic acid (DNA)[1]. Ionizing radiation causes damage to DNA by direct effect and indirect effect. The former is caused by the absorption of energy of ionizing radiation by the DNA molecule itself, the

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later by water radicals generated upon absorption of energy of ionizing radiation by water. On the absorption of energy of ionizing radiation DNA molecule undergoes a chemical change giving radical cation, which on spontaneous deprotonation gives DNA radical, the chemistry of which is similar to DNA radicals produced by water ($^{\bullet}OH$) radicals. When DNA is subjected to ionizing radiation many different changes can occur in DNA[2], ranging from various kinds of base modifications to single and double strand breaks. Even though sugar radicals are actually responsible for strand break formation in DNA, experimental results clearly indicate that base radicals can contribute significantly via transfer of radical sites from base moiety to sugar moiety[3,4].

In order to mimic and understand the mechanism of direct effect of ionizing radiation on DNA, Bansal and Fessenden[5] have used sulphate radical anion a strong electrophilic radical to create radical cation in uracil and substituted uracils. Ravi Kumar and Adinarayana[6] reported that 5-yl radicals obtained from oxidation of thymine by phosphate radical anion ($PO_4^{2\bullet-}$) have been repaired to a greater extent at about 50 μM of dithiothreitol. Sudha et al.[7,8] reported that 5-yl radicals obtained from oxidation of thymine and uracil by $SO_4^{\bullet-}$ have been repaired to a greater extent at about 50 μM of caffeic acid. It has been reported that a number of biochemical reactions in mammalian systems generate reactive oxygen species that are capable of damaging crucial biomolecules such as DNA, proteins and membrane lipids[9,10]. The major reactive oxygen species generated due to oxidative stress and/or by ionizing radiation are the hydroxyl radical ($^{\bullet}OH$), the superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and peroxy radical ($ROO^{\bullet}$). If these radicals are not effectively scavenged by the antioxidant defense mechanism in the tissues, oxidative stress results[11]. The hydroxycinnamic acid derivatives identified as good antioxidants for the reduction of oxidizing OH adducts of pyrimidines via electron transfer[12]. The rate constants for electron transfer from the hydroxycinnamic acids to the oxidizing OH adducts of cytosine or thymine are reported to be ~10^9 mol^{-1} dm^3 s^{-1}.[12] In this paper, we report the results on the protection of thymidine from sulphate radical anion by caffeic acid. Further an attempt has also been made to evaluate the percentage of scavenging of the $SO_4^{\bullet-}$ and the extent of repair of thymidine radicals by caffeic acid.

EXPERIMENTAL

Thymidine and peroxydisulphate were purchased from E. Merck, while caffeic acid was from Sigma chemicals and used as received. The solutions of caffeic acid, thymidine and peroxydisulphate were always prepared afresh with double distilled water. Stock solutions of thymidine and caffeic acid were always freshly prepared and were deaerated by bubbling nitrogen. The solutions of potassium salt of peroxydisulphate was standardized using cerimetry using ferroin indicator. Peroxydisulphate solution was added to a measured

excess of ferrous ammonium sulphate and back titrated with a standard ceric ammonium sulphate solution as reported by Kapoor et al.[13] At room temperature this reaction is rapid enough for analytical purposes and equivalency of ferrous ion to peroxydisulphate is 2 to 1. Required amounts of caffeic acid was then injected as aqueous solution into the mixture of thymidine and peroxydisulphate solutions present in a specially designed 1 cm path length quartz cuvette, which is suitable for both irradiations in the quantum yield reactor as well as for absorbance measurements. The absorbance measurements were made at 267 nm, which is the λ_{max} of thymidine, on a Hitachi UV-visible spectrophotometer (model 3410). Irradiations, were performed at room temperature (25^{o}C) with medium-pressure mercury lamp using Quantum yield reactor, model QYR-20. The irradiations were interrupted at definite intervals of time and the absorbance were noted from which the rate of reaction and the quantum yields of oxidation are calculated. The light intensity at 254 nm was measured by peroxydisulphate chemical actinometry[14].

RESULTS AND DISCUSSION

N_2 saturated aqueous solutions of the reaction mixture containing thymidine (0.5×10^{-4} moldm^{-3}), peroxydisulphate (4.00×10^{-4} moldm^{-3}) and with varying concentrations of caffeic acid were irradiated and the absorbance at 267 nm (λ_{max} of thymidine) with time were noted Fig. 1. The absorbance of thymidine in the reaction mixture at different intervals of irradiation time have been obtained by subtracting the contribution of absorbance of caffeic acid by carrying out a parallel experiment with caffeic acid alone at the same intervals of time measured under similar experimental conditions of the oxidation of thymidine by sulphate radical anion in the presence of caffeic acid. From these the rates of oxidation of thymidine were calculated from the plots of absorbance versus time using microcal origin computer program on personal computer.

The initial rates of oxidation of thymidine by sulphate radical anion have been found to decrease with increase in [caffeic acid] (Table 3). The quantum yields of oxidation of thymidine were calculated from the rates of oxidation of thymidine by sulphate radical anion and the light intensity absorbed by peroxydisulphate at 254 nm, the wavelength at which peroxydisulphate is activated to sulphate radical anions. The quantum yields of oxidation of thymidine (ϕ_{exptl}) at different [caffeic acid] are presented in Table 3. The ϕ_{exptl} values were found to decrease with increasing concentration of caffeic acid. The substances used in the present work viz., caffeic acid and/or thymidine did not undergo any chemical change on shining the light in the absence of peroxydisulphate. Caffeic acid has molar absorption coefficient 7500 dm^{3}mol^{-1} cm^{-1} and thymidine has 7250 dm^{3}mol^{-1} cm^{-1} at 254 nm wavelength at which peroxydisulphate is activated to $SO_4^{\bullet -}$ radicals. Due to this more light is being

absorbed by caffeic acid and/or thymidine and the concentration of $SO_4^{\bullet-}$ radicals produced from activation of peroxydisulphate should decrease with increase in concentration of caffeic acid and/or thymidine. Contrary to this the quantum yields of oxidation of caffeic acid and/or thymidine were found to increase with increase in concentration of caffeic acid and/or thymidine (Table 1 and 2). These results suggest that the excited states of caffeic acid and/or thymidine subsequently transfer energy to peroxydisulphate to give $SO_4^{\bullet-}$ radicals by acting as sensitizers. Thus the efficiency of production of $SO_4^{\bullet-}$ radicals increase, which increases the quantum yields of oxidation of caffeic acid and/or thymidine.

Table 1: Rates of photooxidation of caffeic acid in presence of peroxydisulphate (PDS) at various [caffeic acid] in aqueous anoxic solution

[PDS] = 4.00 x 10^{-4} mol dm^{-3}, Temp. = 298 K
pH = 7.5, Light intensity = 1.01 x 10^{15} quanta s^{-1}

10^5 x [Caffeic acid] (mol dm^{-3})	10^8 x Rate (mol dm^{-3} s^{-1})	Quantum yield
5.00	1.5	2.10
2.00	1.4	0.802
1.00	1.3	0.384

Table 2: Rates of photooxidation of thymidine in presence of peroxydisulphate (PDS) at various [thymidine] in aqueous anoxic solution

[PDS] = 4.00 x 10^{-4} mol dm^{-3}, Temp. = 298 K
pH = 7.5, Light intensity = 1.01 x 10^{15} quanta s^{-1}

10^5 x [Thymidine] (mol dm^{-3})	10^8 x Rate (mol dm^{-3} s^{-1})	Quantum yield
5.00	10.0	6.95
2.00	10.6	3.04
1.00	10.4	1.58

Therefore, in the present work, we proposed that caffeic acid as well as thymidine act as sensitizers and transfers energy to peroxydisulphate to create $SO_4^{\bullet-}$ radicals. This type of sensitization effect was proposed in similar systems earlier[15]. Since, in this system there is competition between thymidine and caffeic acid for $SO_4^{\bullet-}$, the relative amounts of $SO_4^{\bullet-}$ reacting with thymidine decreases with increasing [Caffeic acid]. The rate constant of the

reaction of the sulphate radical anion with thymidine and caffeic acid was reported[16,17] to be 2.2 x 10^9 $dm^3mol^{-1}s^{-1}$ and 1.24 x 10^{10} $dm^3mol^{-1}s^{-1}$, respectively.

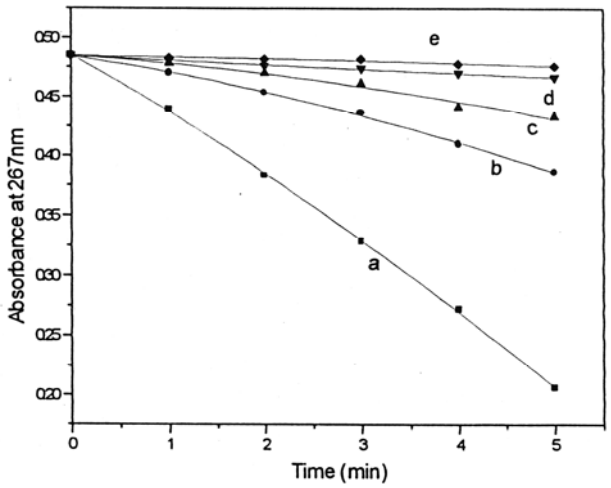


Fig. 1: Effect of caffeic acid on the photooxidation of thymidine by PDS [Thymidine] = 5.00 x 10^{-5} mole dm^{-3}; Caffeic acid = (a) 0.00 (b) 2.00 x 10^{-5} mole dm^{-3} (c) 5.00 x 10^{-5} mol dm^{-3} (d) 1.00 x 10^{-4} mol dm^{-3} and (e) 1.5 x 10^{-4} mol dm^{-3}

The probability of $SO_4^{\bullet-}$ radicals reacting with thymidine {p ($SO_4^{\bullet-}$+ thymidine)} is calculated using the following equation.

$$p(SO_4^{\bullet-} + \text{thymidine}) = \frac{[\text{Thymidine}]k_{\text{thymidine}}}{[\text{Thymidine}]k_{\text{thymidine}} + [\text{caffeic acid}]k_{\text{caffeic acid}}} \quad \ldots(1)$$

$k_{thymidine}$ and $k_{caffeic\ acid}$ are the rate constants of $SO_4^{\bullet-}$ with thymidine and caffeic acid, respectively. If caffeic acid scavenges only $SO_4^{\bullet-}$ radicals and does not give rise to any other reaction (e. g. repair) the ϕ_{exptl} at each [caffeic acid] should be given by equation (2).

$$\phi_{cal} = \phi^0_{exptl} \times p \quad \ldots(2)$$

Where ϕ^0_{exptl} is the quantum yield of oxidation of thymidine in the absence of caffeic acid, and p is the probability given by equation (1). The ϕ_{cal} values at different caffeic acid concentrations are presented in Table 3. It is clear from the data in Table 3 that the calculated quantum yield values (ϕ_{cal}) are larger than the experimentally measured quantum yield values (ϕ_{exptl}). The difference in ϕ_{cal} and ϕ_{exptl} values is proposed to be due to the

prevention of chromophore loss by H atom donation to thymidine radicals by caffeic acid. From the rate constant of sulphate radical anion with caffeic acid, the fraction of $SO_4^{\bullet-}$ radicals scavenged by caffeic acid (Percentage scavenged = (1 – p) x 100) at different [caffeic acid] were calculated (Table 3). These values were a measure of protection of thymidine due to scavenging of $SO_4^{\bullet-}$ radicals by caffeic acid. Table 3 also contains the ϕ^1 values, which are experimentally found quantum yield values at each caffeic acid concentration corrected for sulphate radical anion scavenging by caffeic acid.

$$\phi' = \frac{\phi_{exptl}}{p} \qquad \ldots(3)$$

The ϕ^1 values represent the experimentally found quantum yield values if no scavenging of $SO_4^{\bullet-}$ radicals by caffeic acid occurs and hence, in the absence of repair of thymidine radicals by caffeic acid, ϕ^1 values should all be equal to ϕ^0_{exptl} The observed decrease in the ϕ^1 values with increasing caffeic acid concentration (Table 3) indicates the occurrence of repair of thymidine radicals. The fraction of oxidation of thymidine inhibited by repair of thymidine radicals is given by equation (4).

$$\text{Percentage repair} = \frac{\left(\phi^0{}_{exptl} - \phi'\right)}{\phi^0{}_{exptl}} \times 100 \qquad \ldots(4)$$

The data on percentage repair is presented in Table 3.

Table 3: Effect of [Caffeic acid] on the quantum yields of photooxidation of thymidine in presence of peroxydisulphate (PDS) under anoxic conditions

Light intensity = 1.01 x 10^{15} quanta s^{-1}, [PDS] = 4.00 x 10^{-4} mol dm^{-3},
[Thymidine] = 5.00 x 10^{-5} mol dm^{-3}, pH~7.5, Temp. = 298 K

S. No.	10^5 x [Caffeic acid]	10^8 x rate (mol dm^{-3} s^{-1})	ϕ_{exptl}	P	ϕ_{cal}	ϕ'	% Scavenging	% Repair
1	0.0	10.0	7.00	1.00	7.00	7.00	0.00	0.00
2	2.00	2.60	1.85	0.297	2.08	6.22	70.3	11.0
3	5.00	1.26	0.90	0.144	1.00	6.25	85.6	11.0
4	10.0	0.650	0.448	0.078	0.546	5.74	92.2	18.0
5	15.0	0.31	0.214	0.053	0.371	4.03	94.7	42.4

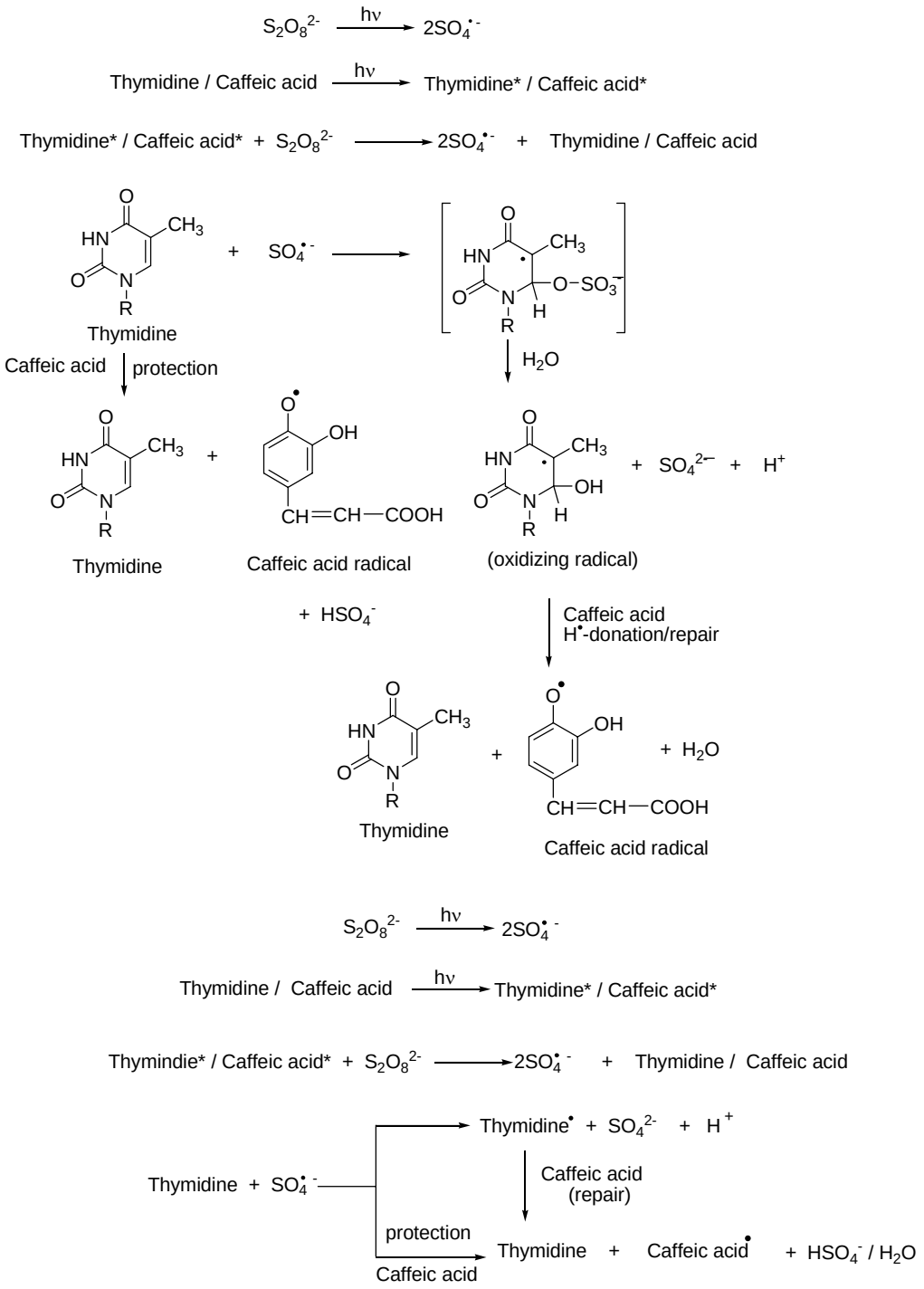
The experimentally determined quantum yield values (ϕ_{exptl}) are lower than the quantum yield values (ϕ_{cal}) calculated using equation (2) under the assumption that caffeic acid acts only as a $SO_4^{\bullet-}$ radical scavenger. This shows that caffeic acid is acting not only as an efficient scavenger of $SO_4^{\bullet-}$ but also acts as an agent for the repair of thymidine radicals. It is therefore obvious that caffeic acid is reacting not only with $SO_4^{\bullet-}$ radicals but also with thymidine radicals. Behrens et al.[18] studied the reaction of $SO_4^{\bullet-}$ with substituted thymines by pulse radiolysis technique and they observed the formation of only C(6)-OH adduct radicals. This is attributed to the reason that C-5 position of thymidine is not accessible for electrophillic addition of $SO_4^{\bullet-}$ due to steric hindrance of methyl group. From the results obtained in the present work (Table 3) indicated that the thymidine radicals, which are oxidizing in nature are efficiently repaired by caffeic acid to the extent of 42.4% at about 150 μM of [Caffeic acid]. The scheme of reactions of protection of thymidine and repair of thymidine radicals through H donation by caffeic acid is given in scheme.

In order to understand the site of attack of $SO_4^{\bullet-}$ on pyrimidine nucleoside i. e. at the base/sugar moiety, a quantitative estimation of the base and sugar moieties present in the nucleoside has been made simultaneously and independently under kinetic conditions at different irradiation times. The results indicate that the sugar moiety is not significantly affected during the oxidation either in the absence or presence of caffeic acid. The rate of oxidation of D-ribose by $SO_4^{\bullet-}$ is lower than the rate of oxidation of nucleoside under the same experimental conditions (Table 4). Further, the rates of oxidation of thymidine by $SO_4^{\bullet-}$ are comparable to those of the rates of oxidation of thymine (Table 4). These results indicate that the base moiety is preferentially attacked by $SO_4^{\bullet-}$ during the oxidation of thymidine. Therefore, the protection and repair offered by caffeic acid is thought to be mainly against base moiety oxidation.

Table 4: Rates of photooxidation of thymine, D-ribose and thymidine in presence of peroxydisulphate (PDS) under anoxic conditions

[PDS] = 4.00 x 10^{-4} mol dm^{-3}, [Substrate] = 5.00 x 10^{-5} mol dm^{-3}
Light intensity = 1.01 x 10^{15} quanta s^{-1}, pH ~ 7.5, temp = 298 K

Substrate	10^8 x initial rate (mol dm^{-3} s^{-1})
Thymine	4.20
Thymidine	10.0
D-ribose	0.163


S2O8 2- hν 2SO4 •-
Thymidine / Caffeic acid hν Thymidine* / Caffeic acid*
Thymidine* / Caffeic acid* + S2O8 2- 2SO4 •- + Thymidine / Caffeic acid
Thymidine
Caffeic acid protection
H2O
Thymidine
Caffeic acid radical
(oxidizing radical)
+ SO4 2- + H+
+ HSO4 -
Caffeic acid
H•-donation/repair
Thymidine
Caffeic acid radical
+ H2O
S2O8 2- hν 2SO4 •-
Thymidine / Caffeic acid hν Thymidine* / Caffeic acid*
Thymindie* / Caffeic acid* + S2O8 2- 2SO4 •- + Thymidine / Caffeic acid
Thymidine + SO4 •-
Thymidine• + SO4 2- + H+
Caffeic acid
(repair)
protection
Caffeic acid
Thymidine + Caffeic acid• + HSO4 - / H2O

Scheme

CONCLUSION

Oxidation studies of thymidine in presence of various [Caffeic acid] and peroxydisulphate have been carried out under different experimental conditions. From competition kinetic studies of thymidine and caffeic acid for $SO_4^{\bullet-}$ percentage of protection of thymidine from $SO_4^{\bullet-}$ with caffeic acid has been calculated. From the experimental quantum yield values (ϕ_{exptl}) and the calculated quantum yield values (ϕ_{cal}) assuming that caffeic acid acts as a scavenger of $SO_4^{\bullet-}$, the percentage repair of thymidine radicals is found to be 42.4%.

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