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Research Article

Kinetics and Mechanism of Protection of URIDINE-5´-MONOPHOSPHATE from Sulphate Radical Anion by Caffeic acid Under Anoxic Conditions

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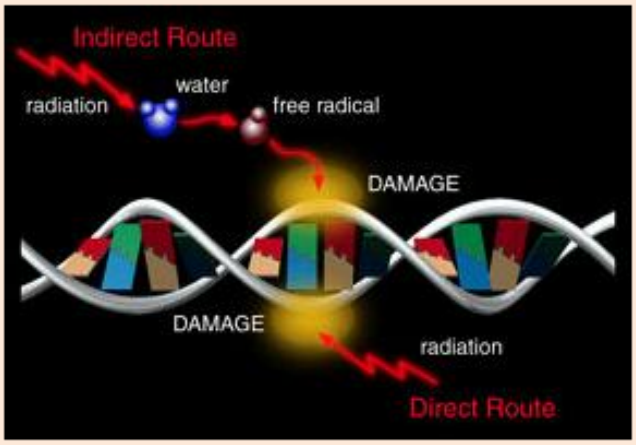
Abstract

The oxidation of uridine-5´-monophosphate by sulphate radical anion ($SO_4^{\bullet-}$) has been followed by measuring the absorbance of uridine-5´-monophosphate at 262nm spectrophotometrically. The rates and the quantum yields (ϕ) of oxidation of uridine-5´-monophosphate 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 uridine-5´ -monophosphate suggesting that caffeic acid acts as an scavenger of $SO_4^{\bullet-}$ and protects uridine-5´-monophosphate from it. Sulphate radical anion competes for uridine-5´-monophosphate as well as for caffeic acid. From the results of experimentally determined quantum yields (ϕ_{exptl}) of oxidation of uridine-5´-monophosphate in presence of different concentrations of caffeic acid and the quantum yields calculated (ϕ_{cal}) $\phi_{cal} = \phi^0_{exptl} \times p$, p is the probability of $SO_4^{\bullet-}$ reacting with uridine-5´-monophosphate in presence of caffeic acid and ϕ^0_{exptl} is the quantum yield of oxidation of uridine-5´-monophosphate in the absence of caffeic acid, assuming caffeic acid acting only as a scavenger of $SO_4^{\bullet-}$ radicals show that ϕ_{cal} values are lower than ϕ_{exptl} values. This observation indicates that caffeic acid might not be able to scavenge $SO_4^{\bullet-}$ as expected, and uridine-5´-monophosphate radicals may be competing for $SO_4^{\bullet-}$ and thus reducing the scavenging capacity of caffeic acid. These observations suggest that the uridine-5´-monophosphate radicals are totally reducing in nature, unlike transient radicals produced in case of uracil, thymine, thymidine, adenine and adenosine reaction with $SO_4^{\bullet-}$. The oxidation of D- Ribose by sulphate radical anion ($SO_4^{\bullet-}$) has been followed by measuring the absorbance of D- Ribose at 480nm spectrophotometrically using phenol sulphuric acid method. The oxidation of D- Ribose by $SO_4^{\bullet-}$ is one order of magnitude lower than the rate of oxidation of uridine-5´-monophosphate. Independent estimation of the sugar moiety in uridine-5´-monophosphate at different times also shows that sugar moiety is not oxidized considerably. Further rate of oxidation of uracil under similar condition is closer to uridine-5´-monophosphate. These results therefore indicate that the base moiety might be the site of attack by the sulphate radical anion in uridine-5´ -monophosphate.



Keywords: Oxidation of caffeic acid, protection of uridine-5´- monophosphate by caffeic acid, Oxidation by sulphate radical anion.

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Introduction

The primary events leading to DNA damage occur either from direct ionization of DNA itself or due to attack of radicals derived from the ionization of the immediate environment [1, 2]. These radicals are mainly formed from water and the most damaging of them is the hydroxyl radical [3]. The study of the direct ionization process within DNA is hampered by the high yields of radicals formed on radiolysis of dilute aqueous solutions of DNA. One of the approaches to study the properties of radical species produced by the direct ionization of homopolynucleotides or DNA in aqueous solutions is by utilizing a strong one-electron oxidant such as $SO_4^{\bullet-}$. In polynucleotides and DNA strand breakage has been reported to be induced within 100μs on reaction of $SO_4^{\bullet-}$ [4, 5]. The reactions of photolytically generated $SO_4^{\bullet-}$ with DNA model compounds [6, 7] have been reported to generate base radicals which subsequently abstract hydrogen from the sugar moiety, thus transferring the radical site, and sugar radicals so formed initiate strand breakage in polynucleotides or DNA. In order to understand the mechanism of damage to DNA and poly nucleotides by free radicals information about the nature and behaviour of the DNA base radicals is desirable. The reactions of $SO_4^{\bullet-}$ with pyrimidine bases and nucleosides along with the protection and repair reactions by caffeic acid are reported [8-11]. It has been observed that caffeic acid acts as an effective protecting agent against damage by $SO_4^{\bullet-}$ [8-11]. It is in this back ground we have carried out kinetic studies of photooxidation of uridine-5´-monophosphate in the absence and presence of caffeic acid to understand the site of attack of sulphate radical anion on uridine-5´-monophosphate and to characterize the nature of transient radicals produced. It is also of interest to evaluate the extent of protection offered by caffeic acid.

Experimental

Uridine-5´-monophosphate and peroxydisulphate were purchased from E.Merck, while caffeic acid was from Sigma chemicals and used as received. The solutions of caffeic acid, uridine-5´-monophosphate and peroxydisulphate were always prepared afresh with double distilled water. Stock solutions of uridine-5´ -monophosphate and caffeic acid were always freshly prepared and were deaerated by bubbling nitrogen. The solutions of potassium salt of peroxydisulphate were 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. [12]. 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 uridine-5´-monophosphate 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 262 nm, which is the λ_{max} of uridine-5´-monophosphate on a HITACHI UV-Visible spectrophotometer (model 3410). Irradiations were performed at room temperature (25^oC) with medium-pressure mercury lamp using Quantum yield reactor model QYR-20. The irradiations were interrupted at definite intervals of time and the absorbance was 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 [13].

Results and Discussion

N_2 saturated aqueous solutions of the reaction mixture containing uridine-5´-monophosphate ($0.5x10^{-4}$ $moldm^{-3}$), peroxydisulphate ($4.00\ x10^{-4}$ $moldm^{-3}$) and with varying concentrations of caffeic acid were irradiated and the absorbance at 262 nm (λ_{max} of uridine-5´ -monophosphate) with time were noted . The absorbance of uridine-5´-monophosphate 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 uridine-5´ -monophosphate by sulphate radical anion in the presence of caffeic acid. From these the rates of oxidation of uridine-5´-monophosphate were calculated from the plots of absorbance versus time using microcal origin computer program on personal computer. The initial rates of oxidation of uridine-5´-monophosphate by sulphate radical anion have been found to decrease with increase in [caffeic acid] (**Table 1**). The quantum yields of oxidation of uridine-5´ -monophosphate were calculated from the rates of oxidation of uridine-5´-monophosphate 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 uridine-5´-monophosphate (ϕ_{exptl}) at different [caffeic acid] are presented in Table 1. 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 uridine-5´-monophosphate did not undergo any chemical change on shining the light in the absence of peroxydisulphate. Caffeic acid has molar absorption coefficient 7500 $dm^3mol^{-1}cm^{-1}$ and uridine-5´-monophosphate has 8800 $dm^3mol^{-1}cm^{-1}$ 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 uridine-5´-monophosphate and the concentration of $SO_4^{\bullet-}$ radicals produced from activation of peroxydisulphate should decrease with increase in concentration of caffeic acid and/or uridine-5´-monophosphate. Contrary to this the quantum yields of oxidation of caffeic acid and/or uridine-5´-monophosphate were found to increase with increase in concentration of caffeic acid [10] and/or uridine-5´-monophosphate (**Table 2**). These results suggest that the excited states of caffeic acid and/or uridine-5´-monophosphate 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 increases, which increases the quantum yields of oxidation of caffeic acid and/or uridine-5´-monophosphate.

Table 1 Effect of [caffeic acid] on the quantum yields of photooxidation of Uridine-5´-monophosphate in presence of peroxydisulphate (PDS) under anoxic conditions

S.No	10^5 x [caffeic acid] (mol dm^{-3})	10^8 x rate (mol dm^{-3} s^{-1})	ϕ_{exptl}	p	ϕ_{cal}	% Scavenging
1	0.00	8.3	7.00	1.00	7.00	0.00
2	1.00	5.75	4.78	0.630	4.41	37.0
3	2.00	4.20	3.50	0.460	3.22	54.0
4	5.00	3.38	2.80	0.254	1.78	74.6

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

Table 2 Rates of photooxidation of Uridine-5´-monophosphate in presence of peroxydisulphate (PDS) at various [Uridine -5´-monophosphate] in aqueous anoxic solution

10^5 x [Uridine -5´-monophosphate] (mol dm^{-3})	10^8 x Rate (mol dm^{-3} s^{-1})	Quantum yield
10.00	8.62	14.15
5.00	8.30	7.00
2.00	8.60	3.00

[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}

Therefore in the present work we propose that caffeic acid as well as uridine-5´-monophosphate 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 [14]. Since in this system there is competition between uridine-5´-monophosphate and caffeic acid for $SO_4^{\bullet-}$, the relative amounts of $SO_4^{\bullet-}$ reacting with uridine-5´-monophosphate decreases with increasing [caffeic acid]. The rate constant of the reaction of the sulphate radical anion with caffeic acid was reported [15] to be 1.24x10^{10} $dm^3mol^{-1}s^{-1}$. The rate constant of the reaction of the sulphate radical anion with uridine-5´-monophosphate has been calculated by the uridine-5´-monophosphate competition method, which is very similar to the one choosen by Akhalaq et al [16] to determine the rate constant for the reaction of OH radicals with polyhydric alcohols in competition with KSCN. In the photolysis experiment, oxygen free N_2 –saturated solutions containing uridine-5´-monophosphate and varying amounts of caffeic acid were irradiated for 4 minutes and decrease of absorbance of uridine-5´-monophosphate was measured. The decrease of absorbance of uridine-5´-monophosphate reflects the number of sulphate radical anions that have reacted with uridine-5´ -monophosphate. From the rate constant of reaction of caffeic acid with $SO_4^{\bullet-}$ ($k_{\text{caffeic acid}+\ SO_4^{\bullet-}}$ = 1.24x10^{10} $dm^3mol^{-1}s^{-1}$), The rate constant of $SO_4^{\bullet-}$ with uridine-5´-monophosphate ($k_{\text{uridine-5´ -monophosphate}+\ SO_4^{\bullet-}}$) can be calculated using equation (1).

$$\frac{[\text{Absorbance of uridine-5´-monophosphate}]_0}{[\text{Absorbance of uridine-5´-monophosphate}]_{\text{caffeic acid}}} = 1 + \frac{k_{(SO4^{\bullet -} + \text{caffeic acid})}[\text{caffeic acid}]}{k_{(SO4^{\bullet -} + \text{uridine-5´-monophosphate})}[\text{uridine-5´-monophosphate}]} - (1)$$

Where [Absorbance of uridine-5´-monophosphate]$_0$ and [Absorbance of uridine-5´-monophosphate]$_{\text{caffeic acid}}$ indicate the decrease in the absorbance of uridine-5´-monophosphate in the absence and presence of caffeic acid respectively, in the same interval of time. Experiments of this kind can be carried out with great accuracy. The rate constant for the reaction of sulphate radical anion with uridine-5´-monophosphate has been calculated with five different concentrations of caffeic acid and average value obtained is 4.15 x10^{9}dm^{3}mol^{-1}s^{-1}.

The probability of $SO_4^{\bullet -}$ radicals reacting with uridine-5´-monophosphate {p($SO_4^{\bullet -}$ + uridine-5´-monophosphate)} is calculated using the following equation.

$$p(SO_4^{\bullet -} + \text{uridine-5´-monophosphate}) = \frac{[\text{Uridine-5´-monophosphate}]k_{\text{uridine-5'-monophosphate}}}{[\text{Uridine-5´-monophosphate}]k_{\text{Uridine-5´-monophosphate}} + [\text{caffeic acid}]k_{\text{caffeic acid}}} - (2)$$

$k_{\text{uridine-5´-monophosphate}}$ and $k_{\text{caffeic acid}}$ are the rate constants of $SO_4^{\bullet -}$ with uridine-5´-monophosphate and caffeic acid respectively. Using the value of ϕ^0_{exptl} (ϕ^0_{exptl} is the quantum yield of oxidation of uridine-5´-monophosphate in the absence of caffeic acid) and p (p is the probability of $SO_4^{\bullet -}$ reacting with uridine-5´ -monophosphate given by Equation (2). We calculated a set of quantum yield values (ϕ_{cal}) using equation (3)

$$\phi_{cal} = \phi^0_{\text{exptl}} \times p - (3)$$

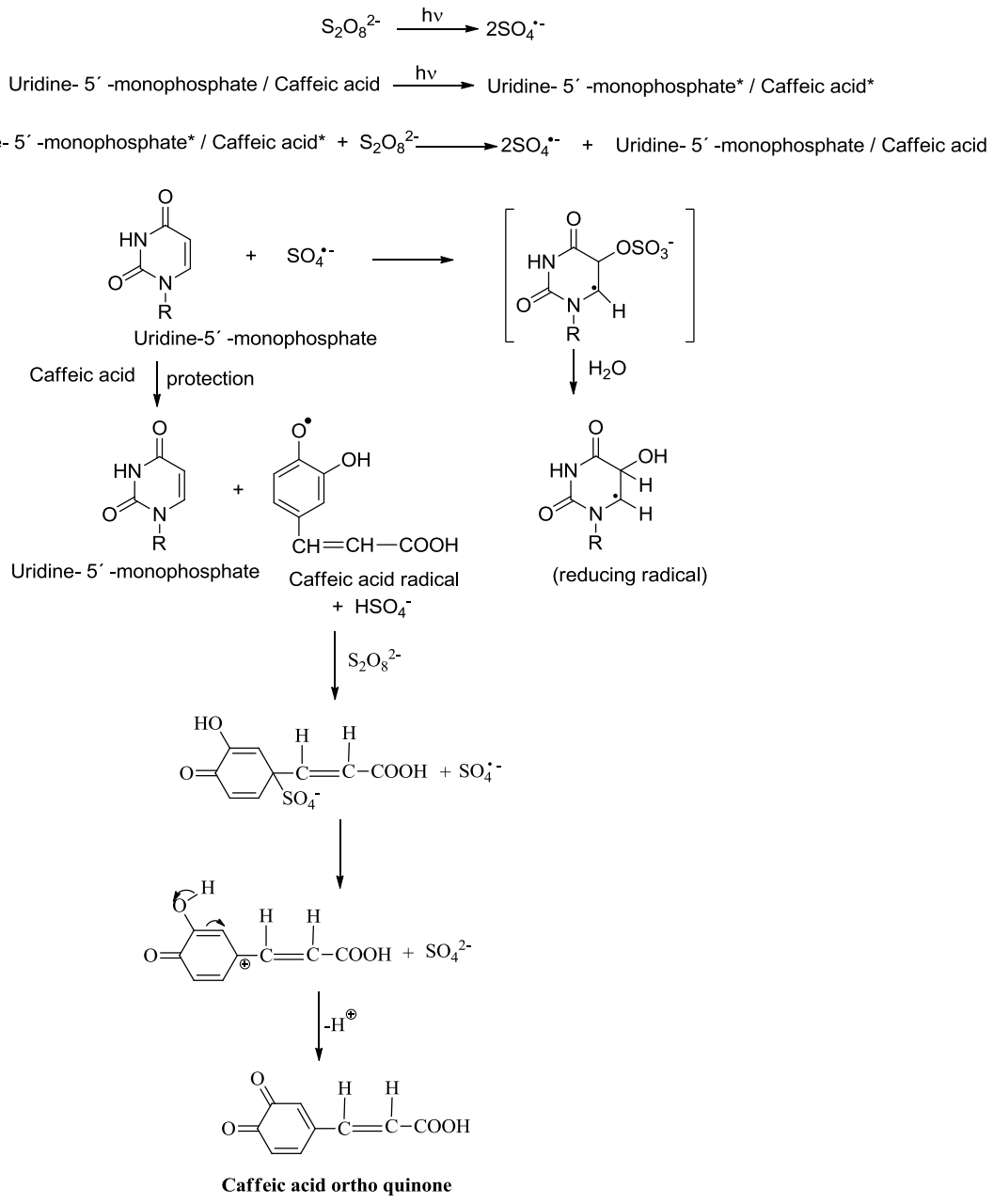
These ϕ_{cal} values represent the quantum yield values for photooxidation of uridine-5´-monophosphate in the presence of caffeic acid corresponding to the situation where role of caffeic acid is restricted only to the scavenging of $SO_4^{\bullet -}$. If caffeic acid is acting as a scavenger alone, ϕ_{cal} are expected to be equal to ϕ_{exptl} values. However it is clear from the data in Table 1 that the calculated quantum yield values (ϕ_{cal}) are smaller than the experimentally measured quantum yield values (ϕ_{exptl}). This observation indicates that caffeic acid might not be able to scavenge $SO_4^{\bullet -}$ as expected and uridine-5´-monophosphate radicals may be competing more for $SO_4^{\bullet -}$ and thus reducing the scavenging capacity of caffeic acid. These observations suggest that the uridine-5´-monophosphate radicals are totally reducing in nature, unlike transient radicals produced in case of thymine, adenine and adenosine reaction with $SO_4^{\bullet -}$, which are oxidizing in nature [8, 17, 18]. It is pertinent to mention that the formation of reducing radicals in a similar system viz., deoxyuridine / $SO_4^{\bullet -}$ has been well reported [7].

From the rate constant of sulphate radical anion with caffeic acid and uridine-5´-monophosphate (Equation (2)), the fraction of $SO_4^{\bullet -}$ radicals scavenged by caffeic acid (Percentage scavenged = (1 – p) x 100) at different [caffeic acid] were calculated (Table 1). These values were a measure of protection of uridine-5´-monophosphate due to scavenging of $SO_4^{\bullet -}$ radicals by caffeic acid.

In the oxidation of pyrimidine nucleosides by OH radicals it has been reported that the base moiety is preferentially oxidized over the sugar moiety [19]. In poly U, it was reported that 93% of OH radicals add to the uracil moiety and only 7% abstract hydrogen atoms from the sugar moiety [20]. In order to understand the site of attack of $SO_4^{\bullet -}$ on uridine-5´-monophosphate i.e. at the base/sugar moiety, a quantitative estimation of the base and sugar moieties present in the nucleotide has been made simultaneously and independently under same 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 uridine-5´-monophosphate under the same experimental conditions (**Table 3**). Further, the rates of oxidation of uridine-5´-monophosphate by $SO_4^{\bullet -}$ are comparable to those of the rates of oxidation of uracil (Table 3). These results indicate that the base moiety is preferentially attacked by $SO_4^{\bullet -}$ during the oxidation of uridine-5´-monophosphate. Therefore, the protection offered by caffeic acid is thought to be mainly against base moiety oxidation. The reactions of protection of uridine-5´-monophosphate are given in **scheme 1**.

Table 3 Rates of photooxidation of uracil, D-ribose, uridine and Uridine-5´-monophosphate in presence of peroxydisulphate (PDS) under anoxic conditions

Substrate	**10^8 x initial rate (mol dm^{-3} s^{-1})**
Uracil	27.2
Uridine	12.0
Uridine -5´-monophosphate	8.30
D-ribose	0.162
[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	



Scheme 1

Conclusions

Oxidation studies of uridine-5´-monophosphate in presence of various [caffeic acid] by sulphate radical anion have been carried out under different experimental conditions. From competition kinetic studies of uridine-5´-monophosphate and caffeic acid for $SO_4^{\bullet-}$, the rate constant of $SO_4^{\bullet-}$ with uridine-5´-monophosphate was calculated and also the percentage of protection of uridine-5´-monophosphate from $SO_4^{\bullet-}$ with caffeic acid has been calculated.

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