Research Article

Kinetics and Mechanism of Protection of Guanosine from Sulphate Radical Anion by Caffeic acid under Anoxic Conditions

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Abstract

The oxidation of guanosine by sulphate radical anion ($SO_4^{\bullet-}$) has been followed by measuring the absorbance of guanosine at 252.5nm spectrophotometrically. The rates and the quantum yields (ϕ) of oxidation of guanosine by $SO_4^{\bullet-}$ have been determined in the presence of different concentrations of caffeic acid. Increase in [caffeic acid] was found to decrease the rate of oxidation of guanosine suggesting that caffeic acid acts as an scavenger of $SO_4^{\bullet-}$ and protects guanosine from it. $SO_4^{\bullet-}$ competes for guanosine as well as for caffeic acid. From the results of experimentally determined quantum yields (ϕ_{exptl}) of oxidation of guanosine in presence of different concentrations of caffeic acid and the quantum yields calculated (ϕ_{cal}), $\phi_{cal} = \phi_{exptl}^0 \times p$, p is the probability of $SO_4^{\bullet-}$ reacting with guanosine in presence of caffeic acid and ϕ^0_{exptl} is the quantum yield of oxidation of guanosine in the absence of caffeic acid, assuming that caffeic acid is acting only as a scavenger of $SO_4^{\bullet-}$, show that ϕ_{cal} values are similar to ϕ_{exptl} values. This observation indicates that role of caffeic acid is restricted only to scavenge $SO_4^{\bullet-}$ and caffeic acid could not be able to repair guanosine radicals produced on reaction with $SO_4^{\bullet-}$. These observations suggest that the guanosine 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 $SO_4^{\bullet-}$ 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 guanosine. Independent estimation of the sugar moiety in guanosine at different times also shows that sugar moiety is not oxidized considerably.

Further rate of oxidation of guanine under similar condition is closer to guanosine. These results therefore indicate that the base moiety might be the site of attack by $SO_4^{\bullet-}$ in guanosine.
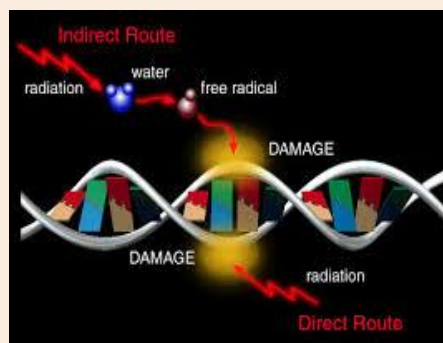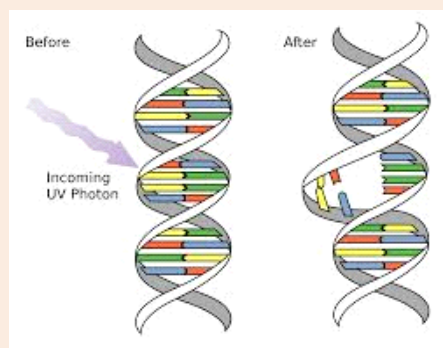
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Introduction

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 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. However, it is possible by chemical methods to mimic the direct effect of radiation in aqueous solutions using strong oxidizing species such as sulphate radical anion ($SO_4^{\bullet-}$) generated in situ by photolysis of peroxydisulphate at 254nm. 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].

Candeias and Steenken[5] have studied the reactions of the nucleosides, Viz., deoxyguanosine, guanosine and 1-methylguanosine with $SO_4^{\bullet-}$ radicals in aqueous solutions and have reported the formation of nucleoside radical cations in the initial step. It has been found that the rate constants for the reaction of $SO_4^{\bullet-}$ radicals with nucleosides [6,7] (k~10^9 dm^{3}mol^{-1}s^{-1}) are significantly higher than those for the abstraction of H atom from alcohols and ethers (k~10^6- 10^8 dm^{3}mol^{-1}s^{-1}). Due to the electron–withdrawing effect of nucleobases further lower rates are expected for the H-abstraction from the sugar moiety of nucleosides by $SO_4^{\bullet-}$ radicals. It has therefore been concluded that $SO_4^{\bullet-}$ reacts predominantly with the base moiety of the nucleosides.

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 [8,9]. 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$_2$O$_2$) and peroxy radical ($ROO^\bullet$). If these radicals are not effectively scavenged by the antioxidant defense mechanism in the tissues, oxidative stress results [10]. The hydroxycinnamic acid derivatives identified as good antioxidants for the reduction of oxidizing OH adducts of pyrimidines (5-yl radicals) via electron transfer [11]. The rate constants for electron transfer from the hydroxycinnamic acids to the oxidizing OH adducts of cytosine or thymine (5-yl radicals) are reported to be ~10^9 mol^{-1} dm^3 S^{-1} [11]. It is also reported [12,13] that caffeic acid efficiently repairs the oxidizing radicals produced in the oxidation of adenine and adenosine by $SO_4^{\bullet-}$ radicals.

In this paper we report the results on the protection of guanosine from sulphate radical anion by caffeic acid. From the competition kinetic studies of $SO_4^{\bullet-}$ radicals with guanosine and caffeic acid, the rate constant of $SO_4^{\bullet-}$ reaction with guanosine has been evaluated. Further an attempt has also been made to evaluate the percentage of scavenging of the $SO_4^{\bullet-}$ radicals by caffeic acid and to characterize the nature of transients produced on reaction of $SO_4^{\bullet-}$ radicals with guanosine.

Experimental

Guanosine and peroxydisulphate were purchased from E.Merck, while caffeic acid was from Sigma chemicals and used as received. The solutions of caffeic acid, guanosine and peroxydisulphate were always prepared afresh with double distilled water. Stock solutions of guanosine and caffeic acid were always freshly prepared and were deaerated by bubbling nitrogen (Anoxic conditions). 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. [14]. 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 guanosine 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 252.5 nm, which is the λ$_{max}$ of guanosine, on a HITACHI UV-visible spectrophotometer (model 3410). Irradiations were performed at room temperature (25°C) with medium-pressure mercury lamp using Quantum yield reactor, model QYR-20. The irradiations were interrupted at definite intervals of time and the absorbances 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 [15].

Results and Discussion

N_2 saturated aqueous solutions of the reaction mixture containing guanosine (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 252.5 nm (λ_{max} of guanosine) with time were noted (**Fig.1**) The absorbance of guanosine 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 guanosine by sulphate radical anion in the presence of caffeic acid. From these the rates of oxidation of guanosine were calculated from the plots of absorbance versus time using microcal origin computer program on personal computer (**Table.1**).
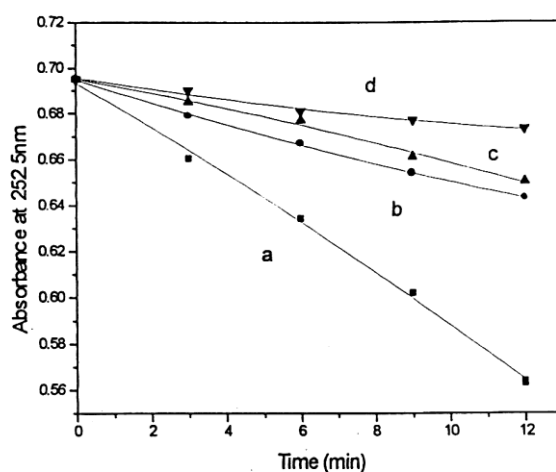

Figure 1 Effect of caffeic acid on the photooxidation of guanosine by peroxydisulphate
(a) [guanosine] = 5.00 x 10^{-5} mol dm^{-3}, caffeic acid = 0.00
(b) [guanosine] = 5.00 x 10^{-5} mol dm^{-3} , caffeic acid = 1.00 x 10^{-6} mol dm^{-3}
(c) [guanosine] = 5.00 x 10^{-5} mol dm^{-3} , caffeic acid = 2.00 x 10^{-6} mol dm^{-3}
(d) [guanosine] = 5.00 x 10^{-5} mol dm^{-3} , caffeic acid = 5.00 x 10^{-6} mol dm^{-3}

The initial rates of oxidation of guanosine by $SO_4^{\bullet-}$ have been found to decrease with increase in [caffeic acid]. (**Table.1**). The quantum yields of oxidation of guanosine were calculated from the rates of oxidation of guanosine by $SO_4^{\bullet-}$ and the light intensity absorbed by peroxydisulphate at 254 nm, the wavelength at which peroxydisulphate is activated to $SO_4^{\bullet-}$. The quantum yields of oxidation of guanosine (ϕ_{exptl}) at different [caffeic acid] are presented in **Table.1**.

Table 1 Effect of [caffeic acid] on the quantum yields of photooxidation of guanosine in presence of peroxydisulphate (PDS) under anoxic conditions.

S.No	10^5 x [caffeic acid] (mol dm^{-3})	10^9 x rate (mol dm^{-3} s^{-1})	exptl	p	cal	% Scavenging
1	0.00	13.3	1.69	1.00	1.69	0.00
2	1.00	6.00	0.778	0.456	0.780	54.4
3	2.00	3.80	0.501	0.287	0.500	71.3
4	5.00	1.80	0.243	0.138	0.240	86.2

Light intensity = 1.01 x 10^{15} quanta s^{-1}; [PDS] = 4.00 x 10^{-4} mol dm^{-3};
[guanosine] = 5.00 x 10^{-5} mol dm^{-3}; pH7.5, Temp = 298 K

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 guanosine 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 guanosine has 13600 $dm^3mol^{-1}cm^{-1}$ 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 guanosine and the concentration of $SO_4^{\bullet-}$ radicals produced from activation of peroxydisulphate should decrease with increase in concentration of caffeic acid and/or guanosine. Contrary to this the quantum yields of oxidation of caffeic acid and/or guanosine were found to increase with increase in concentration of caffeic acid and/or guanosine[12]. (**Table 2).**

Table 2 Rates of photooxidation of guanosine in presence of peroxydisulphate (PDS) in aqueous anoxic solution

10^5 x [guanosine] (mol dm^{-3})	10^9 x Rate (mol dm^{-3} s^{-1})	Quantum yield
5.00	13.3	1.69
2.00	13.1	0.690
1.00	13.6	0.370

[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}

These results suggest that the excited states of caffeic acid and/or guanosine 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 guanosine.

Therefore in the present work we propose that caffeic acid as well as guanosine 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 [12]. Since in this system there is competition between guanosine and caffeic acid for $SO_4^{\bullet-}$, the relative amounts of $SO_4^{\bullet-}$ reacting with guanosine decreases with increasing [caffeic acid]. The rate constant of the reaction of the sulphate radical anion with caffeic acid was reported [12] to be 1.24×10^{10} dm^{3}mol^{-1}s^{-1}. The rate constant of the reaction of the $SO_4^{\bullet-}$ radicals with guanosine has been calculated by the guanosine 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 poly hydric alcohols in competition with KSCN. In the photolysis experiment, oxygen free N_2 –saturated solutions containing guanosine and varying amounts of caffeic acid were irradiated for 4 minutes and decrease of absorbance of guanosine was measured. The decrease of absorbance of guanosine reflects the number of $SO_4^{\bullet-}$ radicals that have reacted with guanosine. From the rate constant of reaction of caffeic acid with $SO_4^{\bullet-}$ radical ($k_{caffeic\ acid+\ SO_4^{\bullet-}} = 1.24 \times 10^{10}$ dm^{3}mol^{-1}s^{-1}), The rate constant of $SO_4^{\bullet-}$ with guanosine ($k_{guanosine+\ SO_4^{\bullet-}}$) can be calculated using equation (1).

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

Where [Absorbance of guanosine]$_0$ and [Absorbance of guanosine]$_{guanosine}$ indicate the decrease in the absorbance of guanosine 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 guanosine has been calculated with five different concentrations of caffeic acid and average value obtained is 2.0 $\times 10^8$ dm^{3}mol^{-1}s^{-1}.

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

$$p\,(SO_4^{\bullet-} + Guanosine) = \frac{[\text{Guanosine}]k_{Guanosine}}{[\text{Guanosine}]k_{Guanosine} + [\text{caffeic acid}]k_{caffeic\ acid}} - (2)$$

$k_{guanosine}$ / ($k_{guanosine+}$ $so_4^{\bullet-}$) and $k_{caffeic\ acid}$/ k($_{caffeic\ acid+}$ $so_4^{\bullet-}$) are the rate constants of $SO_4^{\bullet-}$ with guanosine and caffeic acid respectively. Using the value of ϕ^0_{exptl} (ϕ^0_{exptl} is the quantum yield of oxidation of guanosine in the absence of caffeic acid) and p (p is the probability of $SO_4^{\bullet-}$ reacting with guanosine given by Equation (2). We calculated a set of quantum yield values (ϕ_{cal}) using equation (3)

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

These ϕ_{cal} values represent the quantum yield values for photooxidation of guanosine in the presence of caffeic acid corresponding to the situation where role of caffeic acid is restricted only to the scavenging of $SO_4^{\bullet-}$ and not involved in repair of guanosine radicals formed on reaction with $SO_4^{\bullet-}$. If caffeic acid is acting as a scavenger alone, ϕ_{cal} are expected to be equal to ϕ_{exptl} values and It is clear from the data in **Table.1** that the calculated quantum yield values (ϕ_{cal}) are very close to the experimentally measured quantum yield values (ϕ_{exptl}). This observation indicates that role of caffeic acid is restricted only to scavenging of $SO_4^{\bullet-}$ and not involved in repair of guanosine radicals, this suggest that the guanosine 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 [12,13,17] and repaired by caffeic acid efficiently.

O' Neill and co-workers [18-20] found that reaction of $^\bullet OH$ radicals with 2′-deoxyguanosine and its 5′-monophosphate gives 50% oxidizing and 50% reducing type radicals as determined by redox titrations using a variety of reductants and oxidants. When $^\bullet OH$ radicals attack the guanine nucleobase in DNA, Three types of OH-adduct radicals, viz.,C4-OH, C5-OH and C8-OH are formed. The C4-OH and C5-OH adduct radicals revert back to guanine by gaining an electron from the medium [21,22].

According to Pullmann [23] the calculated charge density and localization energy values of elctrophillic attack at various carbon atoms in purine ring suggest that C8 is more favourable for electrophillic attack. Vieira and Steenken [24] studied the reactions of 6- and 9- substituted purines with $^\bullet OH$ radicals by pulse radiolysis and they found that $^\bullet OH$ radicals add at the C(4) and C(8) positions of the purines. The C(4)-OH adduct radical undergoes unimolecular dehydration to give a radical with oxidizing properties, While the C(8)-OH adduct radical undergoes a transformation leading to the opening of the ring. The C(8)-OH adduct radicals have been found to be predominantly reducing in nature. It is reported that attack of $SO_4^{\bullet-}$ on guanosine occurs mainly at C8 position [25].

From the rate constant of $SO_4^{\bullet-}$ with caffeic acid and guanosine (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 guanosine due to scavenging of $SO_4^{\bullet-}$ radicals by caffeic acid.

In order to understand the site of attack of $SO_4^{\bullet-}$ on guanosine 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 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 guanosine under the same experimental conditions **(Table.3)**. Further, the rates of oxidation of guanosine by $SO_4^{\bullet-}$ are comparable to those of the rates of oxidation of guanine **(Table.3)**.

Table 3 Rates of photooxidation of guanine D-ribose and guanosine in presence of peroxydisulphate (PDS) under anoxic conditions

Substrate	10^9 x initial rate (mol dm^{-3} s^{-1})
guanine	17.2
guanosine	13.3
D-ribose	1.63

[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

These results indicate that the base moiety is preferentially attacked by $SO_4^{\bullet-}$ during the oxidation of guanosine. Therefore, the protection offered by caffeic acid is thought to be mainly against base moiety oxidation. The reactions of protection of guanosine are given in **scheme 1.**
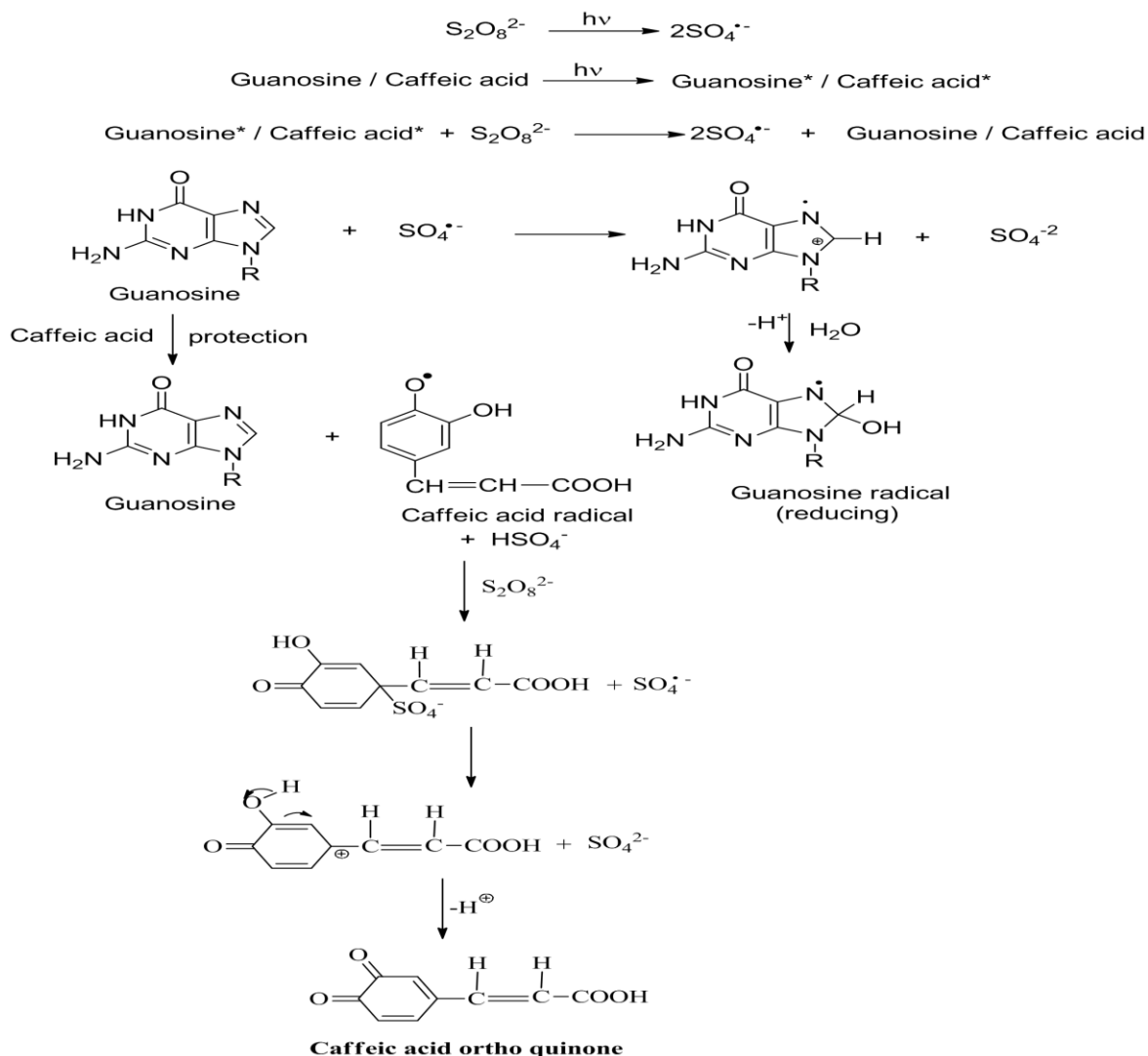
Scheme 1 Scheme of Reactions of Protection of Guanosine by Caffeic acid

Conclusions

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

Acknowledgements

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