Kinetics and mechanism of protection of adenine from sulphate radical anion by caffeic acid under anoxic conditions

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The rates of photo-oxidation of adenine in the presence of peroxydisulphate (PDS) have been determined by measuring the absorbance of adenine at 260.5 nm spectrophotometrically. The rates and the quantum yields (φ) of oxidation of adenine by sulphate radical anion (SO₄⁻) have been determined in the presence of different concentrations of caffeic acid. Increase in the concentration of caffeic acid is found to decrease the rate of oxidation of adenine suggesting that caffeic acid acts as an efficient scavenger of SO₄⁻ and protects adenine from it; SO₄⁻ competes for adenine as well as for caffeic acid. From competition kinetics, the rate constant of SO₄⁻ with caffeic acid has been calculated to be 1.24 ± 0.2 x 10¹⁰ mol⁻¹dm³s⁻¹. The quantum yields of photo-oxidation of adenine have been calculated from the rates of oxidation of adenine and the light intensity absorbed by PDS at 254 nm, the wavelength at which PDS is activated to SO₄⁻. The results of experimentally determined quantum yields (φ_{expt}) and the quantum yields calculated (φ_{cl}) by assuming that caffeic acid acts only as a scavenger of SO₄⁻ radicals show that φ_{expt} values are lower than φ_{cl} values. The φ values, which are experimentally found quantum yield values at each caffeic acid concentration and corrected for SO₄⁻ scavenging by caffeic acid, are also found to be greater than φ_{expt} values. These observations suggest that the adenine radicals are repaired by caffeic acid, in addition to scavenging of sulphate radical anions.

It is known that hydroxycinnamic acids are natural antioxidants and their antioxidant and antifungal activity is mainly due to their ability to scavenge several oxidizing free radicals. In recent times focus is on the protective action of naturally occurring antioxidants and in this connection studies involving caffeic acid assume importance due to its wide spread occurrence in nature. Ionizing radiations are known to cause changes such as base modifications, single and double strand breaks in DNA. Even though sugar radicals are responsible for strand break in DNA, experimental evidence indicates that base radicals also contribute to it by transfer of their radical sites from base moiety to sugar moiety. Strand breaks result in damage to DNA. DNA damage due to ionizing radiation can be a direct or an indirect effect. The former is caused by the absorption of the ionizing radiation by DNA molecule itself, and the latter by water radicals generated upon absorption of the ionizing radiation by water. It is difficult to distinguish experimentally between these two modes of damage to DNA. On absorption of ionizing radiation, DNA molecule undergoes chemical change giving radical cation, which on spontaneous deprotonation gives DNA radical, the chemistry of which is similar to DNA radicals produced by OH radicals. In order to mimic and understand the mechanism of direct effect of ionizing radiation on DNA model compounds and to understand the mechanism of protection from sulphate radical anion (SO₄⁻), we have carried out a systematic kinetic study of oxidation of adenine by SO₄⁻ in the presence of varying concentrations of caffeic acid. In this paper, we report the results on the protection of adenine from SO₄⁻ by caffeic acid. From the competition kinetic studies of SO₄⁻ with adenine and caffeic acid, the rate constant of SO₄⁻ reaction with caffeic acid has been evaluated. Further, an attempt has also been made to evaluate the extent of repair of adenine radicals by caffeic acid.

Materials and Methods
Adenine and peroxydisulphate (PDS) were purchased from E. Merck, Germany and caffeic acid was from Sigma, USA. All solutions were prepared using double distilled water. Stock solutions of adenine and caffeic acid were always freshly prepared and deaerated by bubbling nitrogen. The solutions of potassium salt of peroxydisulphate (PDS) were prepared using double distilled water and standardised by cerimetry using ferroin indicator. PDS solution

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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. At room temperature, this reaction is rapid enough for analytical purposes and equivalency of ferrous ion to PDS is 2 to 1. Required amounts of caffeic acid were then injected as aqueous solution into the mixture of adenine and PDS 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 260.5 nm, which is the $\lambda_{\text{max}}$ of adenine on a HITACHI UV-visible spectrophotometer (model 3410). Irradiations, were performed at room temperature (25$^\circ$C) with high-pressure mercury lamp using quantum yield reactor model QYR-20 and were interrupted at definite intervals of time and the absorbance was noted from which the rate of reaction and the quantum yields of oxidation were calculated. The absorbance at 254 nm was measured by PDS chemical actinometry. In this method, the PDS solution ($2.50 \times 10^{-3}$ mol dm$^{-3}$) was prepared and standardized iodometrically. PDS solution (3.0 ml) was exposed to light in the quantum yield reactor for 10, 20 and 30 min and the concentration of remaining PDS was estimated. From the reported quantum yield of decomposition of PDS (0.60), the light intensity was calculated. The light intensity absorbed by PDS was calculated using the equation:

\[
I_{\text{PDS}} = \frac{\epsilon_{\text{PDS}}[\text{PDS}]}{\epsilon_{\text{PDS}}[\text{PDS}] + \epsilon_{\text{adenine}}[\text{adenine}]} \times I_t, \quad \ldots \ (1)
\]

where, $I_{\text{PDS}}$ is the intensity of light absorbed by PDS in a reaction mixture; $I_t$ is the total intensity of light measured from PDS actinometry; $\epsilon_{\text{PDS}}$ is the molar absorption coefficient of PDS at 254 nm (24.1 dm$^3$ mol$^{-1}$cm$^{-1}$), and $\epsilon_{\text{adenine}}$, the molar absorption coefficient of adenine at 254 nm (12500 dm$^3$ mol$^{-1}$ cm$^{-1}$).

**Results and Discussion**

$N_2$-saturated aqueous solutions of the reaction mixture containing adenine ($0.5 \times 10^{-4}$ mol dm$^{-3}$), PDS ($4.0 \times 10^{-4}$ mol dm$^{-3}$) and caffeic acid were irradiated and the absorbance at 260.5 nm ($\lambda_{\text{max}}$ of adenine) with time was noted. The rates of oxidation of adenine were calculated from the plots of absorbance versus time using a microcal origin computer program on personal computer (Fig. 1). $N_2$-saturated aqueous solutions of reaction mixture containing caffeic acid and PDS were irradiated and the absorbance at 310 nm ($\lambda_{\text{max}}$ of caffeic acid) with time was noted. The rates of oxidation of caffeic acid were calculated from the plots of absorbance versus time using computer program as mentioned above.

The initial rates of oxidation of caffeic acid by $SO_4^{\cdot -}$ at various concentrations of caffeic acid are given in Table 1. The initial rates of oxidation of adenine by $SO_4^{\cdot -}$ have been found to decrease with increase in the concentration of caffeic acid (Table 2, Fig. 1). The quantum yields of oxidation of adenine are calculated from the rates of oxidation of adenine by $SO_4^{\cdot -}$ and the light intensity absorbed by PDS at 254 nm, the wavelength at which PDS is activated to sulphate radical anions. The quantum yields of oxidation of adenine ($\phi_{\text{exptl}}$) at different caffeic acid concentrations are presented in Table 2. The $\phi_{\text{exptl}}$ values are found to decrease with increasing concentration of caffeic acid. Since in this system
there is competition between adenine and caffeic acid for SO₄²⁻, the relative amounts of SO₄²⁻ reacting with adenine decrease with increasing concentration of caffeic acid.

The rate constant of the reaction of the SO₄²⁻ with adenine has been reported to be 4.6 × 10⁹ mol⁻¹ dm³ s⁻¹. The rate constant for the reaction of SO₄²⁻ with caffeic acid has been calculated by the adenine competition method, which is very similar to the one chosen by Akhalaq et al. to determine the rate constant for the reaction of OH radicals with polyhydric alcohols in competition with KSCN. In the photolysis experiment, oxygen-free N₂-saturated solutions containing adenine and varying amounts of caffeic acid were irradiated for six minutes and the decrease of absorbance of adenine was measured. The decrease of absorbance of adenine reflects the number of SO₄²⁻ radical that have reacted with adenine. From the rate constant of reaction of adenine with SO₄²⁻ (k_{adenine} = 4.6 × 10⁹ mol⁻¹ dm³ s⁻¹), the rate constant for the reaction of SO₄²⁻ with caffeic acid (k_{caffeic acid}) can be calculated using equation:

\[
p (\text{SO}_4^{2-} + \text{Adenine}) = \frac{[\text{Adenine}]k_{\text{Adenine}}}{[\text{Adenine}] + [\text{Caffeic acid}]k_{\text{caffeic acid}}}
\]

where, \(k_{\text{adenine}}\) and \(k_{\text{caffeic acid}}\) are the rate constants of SO₄²⁻ with adenine and caffeic acid, respectively. If caffeic acid scavenges only SO₄²⁻ radicals and does not give rise to any other reaction (e.g. repair), the \(\phi_{\text{exptl}}\) at each concentration of caffeic acid may be given by equation:

\[
\phi_{\text{cl}} = \phi_{\text{exptl}}^p \times p \quad \ldots (4)
\]

where, \(\phi_{\text{exptl}}^p\) is the quantum yield of oxidation of adenine in the absence of caffeic acid, and \(p\) is the probability given by Eq. (3).

The calculated quantum yield (\(\phi_{\text{cl}}\)) values at different caffeic acid concentrations are presented in Table 2. It is clear from the data (Table 2) that \(\phi_{\text{cl}}\) values are larger than the experimentally measured quantum yield values (\(\phi_{\text{exptl}}\)). The difference in \(\phi_{\text{cl}}\) and \(\phi_{\text{exptl}}\) values is proposed to be due to the prevention of chromophore loss by H atom donation to adenine radicals by caffeic acid. From the rate constant of SO₄²⁻ with caffeic acid, the fraction of SO₄²⁻ radicals scavenged by caffeic acid (% scavenged = (1 – \(p\)) × 100) at different concentrations of caffeic acid were calculated (Table 2). These values were a measure of protection of adenine due to scavenging of SO₄²⁻ radicals by caffeic acid. Table 2 also contains the \(\phi'\) values, which are experimentally found \(\phi\) values at each caffeic acid concentration corrected for SO₄²⁻ radical scavenging by caffeic acid by equation:

\[
\phi' = \frac{\phi_{\text{exptl}}}{p}
\]

where, \(\phi'\) represents the experimentally found \(\phi\) values if no scavenging of SO₄²⁻ radicals by caffeic acid.
acid occurs and hence, in the absence of repair of adenine radicals by caffeic acid, $\phi'$ values should all be equal to $\phi^0_{\text{exptl}}$. The observed decrease in the $\phi'$ with increasing caffeic acid concentration (Table 2) indicates the occurrence of repair of adenine radicals. The fraction of oxidation of adenine inhibited by repair of adenine radicals is given by equation:

$$
\% \text{Repair} = \left( \frac{\phi^0_{\text{exptl}} - \phi'}{\phi^0_{\text{exptl}}} \right) \times 100 \quad \ldots (6)
$$

The data on percentage repair is presented in Table 2.

The experimentally determined quantum yield values ($\phi_{\text{exptl}}$) are lower than the quantum yield values ($\phi_0$) calculated using eq. (4) under the assumption that caffeic acid acts only as a $\text{SO}_4^{*-}$ radical scavenger. This shows that caffeic acid acts not only as an efficient scavenger of $\text{SO}_4^{*-}$, but also as an agent for the repair of adenine radicals. It is, therefore, obvious that caffeic acid reacts not only with $\text{SO}_4^{*-}$ radicals, but also with adenine radicals. The repair reaction of caffeic acid is explained in terms of the H-donation. The results obtained in the present study (Table 2) indicate that adenine radicals are efficiently repaired by caffeic acid to the extent of ~ 95.55% at about 50 $\mu$M of caffeic acid concentration. The reactions of protection of adenine and repair of adenine radicals is given in Scheme 1.

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**References**