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THE REACTION OF SULFITE RADICAL ANION WITH NUCLEIC ACID COMPONENTS[†]

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The sulfite radical anion ($SO_3^{\cdot -}$) is the first intermediate in the autoxidation of sulfite to sulfate. Using competition kinetics, its reactivities with the nucleic acid bases and the corresponding nucleosides were investigated. The second order rate constants were found to be rather low, $k \leqslant 1 \times 10^6 \, dm^3 mol^{-1} s^{-1}$ at pH 7. As a competitor, the carotenoid crocin was used, which was found to be bleached very efficiently by $SO_3^{\cdot -}$ ($k = 1.0 \times 10^9 \, dm^3 mol^{-1} s^{-1}$).

KEY WORDS: Sulfite radical, nucleic acid bases, nucleosides, crocin, pulse radiolysis, rate constants, competition studies.

INTRODUCTION

Sulfur dioxide is a major air pollutant. Its hydrated form, the sulfite anion, is known to autoxidize rapidly via a chain reaction involving the sulfur containing radicals, $SO_3^{\cdot -}$, $SO_4^{\cdot -}$, and $SO_5^{\cdot -}$.[1] Sulfite has been found to exert damage on DNA under conditions of free radical formation, i.e. in the presence of oxygen.[2] However, the kinetics and mechanisms of such damaging reactions have not been investigated to date. In this study, we present data on the reactivities of several nucleic acid components with $SO_3^{\cdot -}$, which is the first intermediate in the autoxidation of sulfite to sulfate.

MATERIALS AND METHODS

Crocin was isolated from saffron using an established procedure.[3] All other reagents were of the highest purity commercially available and were used as supplied. Water was purified using a Millipore "Milli-Q" system. To prevent sulfite autoxidation, all solutions were prepared in deoxygenated water.

The pulse radiolysis set-up[4] consisted of a Febetron accelerator delivering electron pulses of 1.8 MeV, a Xenon lamp as analyzing light source, a spectrograph, a photomultiplier array, a transient digitizer, and a computer for experiment control and data evaluation.

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RESULTS AND DISCUSSION

The sulfite radical, $SO_3^{\cdot -}$, was produced in aqueous solution by the reaction of sulfite (approximately equimolar amounts of SO_3^{2-} and HSO_3^- at pH 7) with $^{\cdot}OH$ radicals generated by pulse radiolysis:

$$SO_3^{2-} + {}^{\cdot}OH \xrightarrow{k_1} SO_3^{\cdot -} + OH^- \qquad [1]$$

$$HSO_3^- + {}^{\cdot}OH \xrightarrow{k_2} SO_3^{\cdot -} + H_2O \qquad [2]$$

($k_1 = 5.5 \times 10^9 dm^3 mol^{-1} s^{-1}$; $k_2 = 9.5 \times 10^9 dm^3 mol^{-1} s^{-1}$).[1]

$SO_3^{\cdot -}$ has a rather weak absorption spectrum peaking at about 260 nm.[1] Its reactions could not be observed directly because of the low molar absorptivity of the radical and the overlap of its absorption band with those of the substrates. Therefore, we tried to determine rate constants using the method of competition kinetics. Several potential

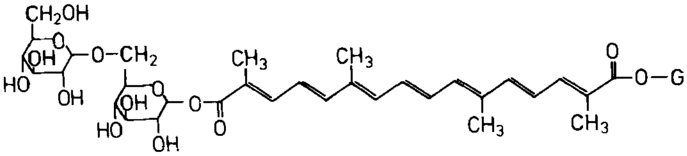

FIGURE 1 Structure of crocin.

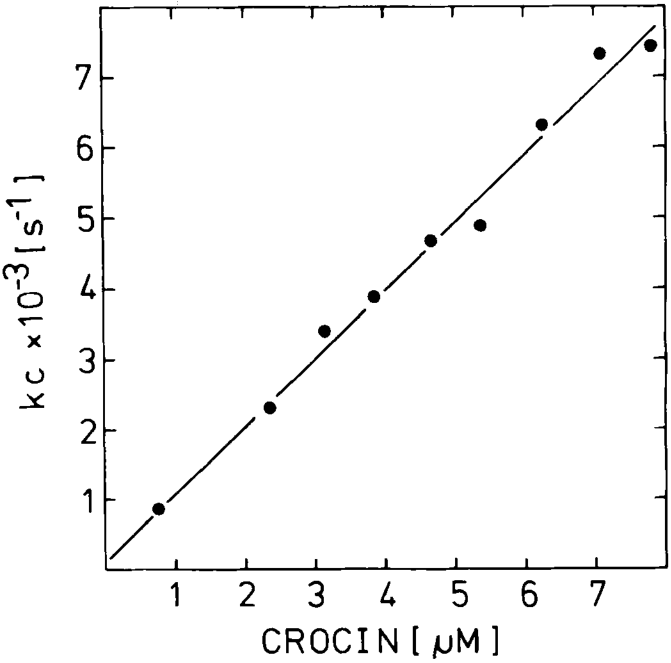

FIGURE 2 Bleaching of crocin by $SO_3^{\cdot -}$. Dependence of pseudo-first order rate constant (*kc*) on crocin concentration.

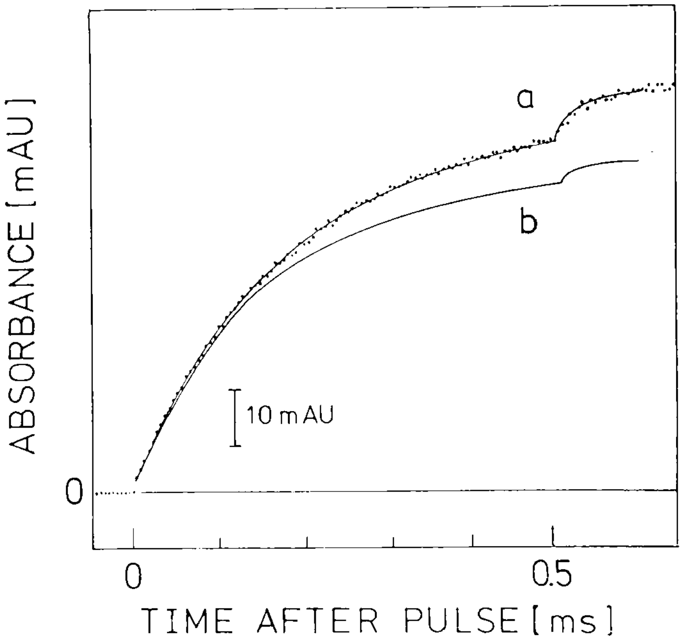

FIGURE 3 Competition experiment with adenine as a substrate. Adenine concentration 1.1 mM, radiation dose 5 Gy. Dots represent experimental points; the solid lines are calculated curves using the following rate constants [$dm^3 mol^{-1} s^{-1}$]: $SO_3^{\cdot -}$ + $SO_3^{\cdot -}$, 1.0×10^9;[1] $SO_3^{\cdot -}$ + crocin, 1.0×10^9; $SO_3^{\cdot -}$ + adenine, (a) 0, (b) 1.0×10^6.

competitors were investigated, of which the carotenoid crocin (structure see Figure 1) was found to be the most suitable at neutral pH.

Crocin is bleached very efficiently by $SO_3^{\cdot -}$. However, at 440 nm – the absorption maximum of crocin – the bleaching kinetics were biphasic, whereas in the 490–500 nm region the kinetics could be evaluated for competition studies using computer simulation.[5] Figure 2 shows a plot of the results obtained at various crocin concentrations; from these data an average rate constant of $1.0 \times 10^9\, dm^3 mol^{-1} s^{-1}$ was determined.

Using crocin as competitor, the reaction of four DNA or RNA bases (adenine, cytosine, thymine, and uracil) and five nucleosides (adenosine, guanosine, cytidine, thymidine, and uridine) with $SO_3^{\cdot -}$ was investigated. Because of its very low solubility in water, guanine could not be studied. Base or nucleoside concentrations of up to 1.8 mM, i.e. a 500-fold excess over the competitor crocin, were used. In all experiments the bleaching kinetics showed no significant deviation from those obtained in the absence of the substrates, as exemplified in Figure 3 for adenine. From the results we calculated an upper limit of the rate constant of the reaction of $SO_3^{\cdot -}$ with these substances to be about $1 \times 10^6\, dm^3 mol^{-1} s^{-1}$. In the case of the nucleosides, the amount of crocin bleached was slightly reduced in a non-competitive manner; however, the kinetics remained unchanged, so it could be ruled out that this effect was due to a reaction of $SO_3^{\cdot -}$ with the nucleosides.

These results demonstrate that DNA damage caused by autoxidizing sulfite probably cannot be attributed to a direct reaction of $SO_3^{\cdot -}$ radicals, even though experimental evidence for $SO_3^{\cdot -}$ addition to uracil derivatives has been reported.[6] Further investigations will be needed to show if the other radicals involved in sulfite autoxidation, namely $SO_5^{\cdot -}$ and $SO_4^{\cdot -}$, react with DNA components more efficiently.

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