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Reactions of Oxidation Intermediates of Sulphite Species with Some Cellular Components of Plants

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ABSTRACT

The aerobic oxidation of sulphite to sulphate via free radical intermediates is briefly reviewed. The co-oxidation of certain biological compounds linked to the aerobic oxidation of sulphite is discussed; in particular, ethylene formation from methional; the oxidation of methionine and tryptophan; the oxidation of indole-3-acetic acid; the destruction of chlorophylls and β-carotene; and lipid peroxidation and membrane damage.*

INTRODUCTION

Sulphite* is widely used in foods, beverages and pharmaceuticals (Schroeter, 1966) and gaseous SO_2 is a major air pollutant which causes injury to vegetation, animals and human beings (US Department of Health, Education and Welfare, 1969). Sulphite is a metabolic intermediate both in the oxidation of organic sulphur to sulphate by mammals and in the reduction of sulphate to organic sulphur by autotrophic organisms. In animal and plant tissues, SO_2 is mainly oxidised to sulphate. There are three mechanisms by which sulphite can be oxidised to sulphate: (1) aerobic oxidation through a free radical mechanism, (2) oxidation by an oxidising agent and (3) oxidation mediated by sulphite oxidase.

* Sulphite is used to designate all dissolved and hydrated species of SO_2, HSO_3^- and SO_3^{2-}.

The aerobic oxidation of sulphite to sulphate through a free radical chain mechanism can be initiated by ultraviolet light (Backstrom, 1927; Hayon *et al.*, 1972), metals (Abel, 1951), illuminated dyes (Fridovich & Handler, 1960; Asada & Kiso, 1973), electrolytic processes (Fridovich & Handler, 1958) and enzymatic reactions (Fridovich & Handler, 1958, 1960; Klebanoff, 1961; Yang, 1967, 1970; Nakamura, 1970), all of which are able to produce free radicals. The available data are in good agreement with the view that O_2^-, $OH\cdot$ and SO_3^- radicals are generated during the aerobic oxidation of sulphite and that these radicals are, in turn, responsible for the propagation of the sulphite–oxygen chain reaction as described by the following schemes (Abel, 1951; Yang, 1970; Hayon *et al.*, 1972):

Initiation:

$$SO_3^{2-} + O_2 \rightarrow SO_3^- + O_2^- \quad (1)$$

Propagation:

$$SO_3^- + O_2 \rightarrow SO_5^- \quad (2)$$

$$SO_5^- + SO_3^{2-} \rightarrow SO_4^- + SO_4^{2-} \quad (3)$$

$$SO_4^- + SO_3^{2-} \rightarrow SO_4^{2-} + SO_3^- \quad (4)$$

$$SO_4^- + OH^- \rightarrow SO_4^{2-} + OH\cdot \quad (5)$$

$$OH\cdot + SO_3^{2-} \rightarrow OH^- + SO_3^- \quad (6)$$

$$O_2^- + SO_3^{2-} + 2H^+ \rightarrow 2OH\cdot + SO_3^- \quad (7)$$

Termination:

$$OH\cdot + SO_3^- \rightarrow OH^- + SO_3 \quad (8)$$

$$SO_3^- + O_2^- + 2H^+ \rightarrow SO_3 + H_2O_2 \quad (9)$$

$$(H_2O_2 + SO_3^{2-} \rightarrow H_2O + SO_4^{2-}$$

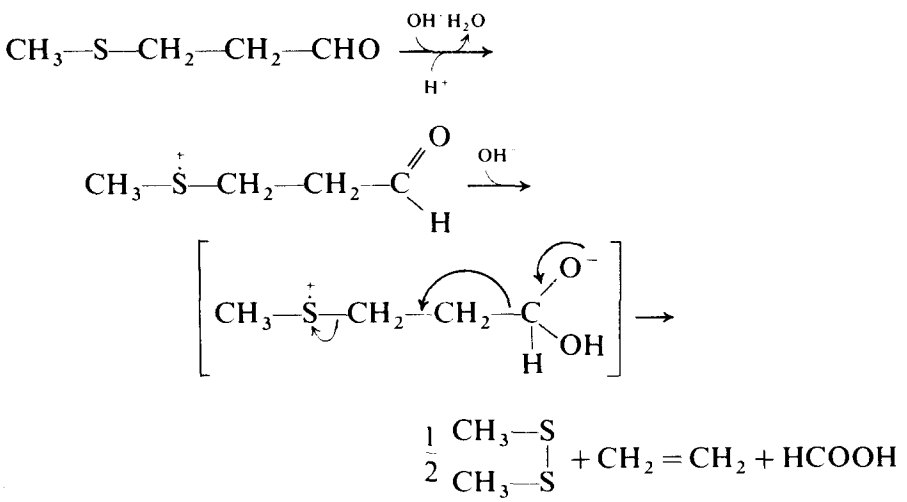
$$SO_3 + H_2O \rightarrow SO_4^{2-} + 2H^+)$$

Once eqn (1) is initiated by metal ions, ultraviolet light, or enzymic reactions, chain propagation reactions of eqns (2) to (7) are maintained with concomitant formation of sulphate. The chain length of the reaction

has been estimated to be 30000 mols per mol O_2^- in the xanthine–xanthine oxidase system (Fridovich & Handler, 1958) and 300 mols in isolated chloroplasts under illumination (Asada & Kiso, 1973). The metal-initiated auto-oxidation of sulphite is inhibited by many reagents, including organic acids, alcohols, thiols, amines and proteins, which are present in abundance in the cells, and thus may serve as radical scavengers (Schroeter, 1966; McCord & Fridovich, 1968). However, in the presence of enzymes such as peroxidase, it has been shown that the chain-initiated sulphite oxidation is not inhibited by these scavengers to a significant extent (Yang, 1970). The oxy and sulphur-oxide radicals generated during the aerobic oxidation of sulphite can function in a number of oxidative reactions of biological importance. In this paper, I summarise our work on the co-oxidation of a number of biological compounds linked to the aerobic oxidation of sulphite.

ETHYLENE FORMATION FROM METHIONAL

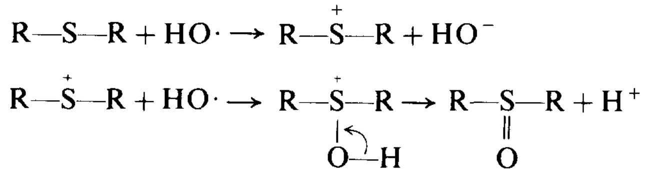
Ethylene is rapidly and efficiently formed from 3-(methylthio)-propionaldehyde (methional) or from 4-(methylthio)-2-oxobutanoic acid (Yang, 1967, 1969) by peroxidase during the oxidation of sulphite. Presumably, OH· and O_2^-, generated during the peroxidase-catalysed sulphite oxidation, are responsible for the reactions shown by the following schemes:



Later, Beauchamp & Fridovich (1970) ascertained that it was OH· which was responsible for the ethylene formation from methional. The formation of ethylene from methional has since been used as a diagnostic test for the generation of OH radical in the biological systems.

OXIDATION OF METHIONINE AND TRYPTOPHAN

Methionine and other thioethers are rapidly and efficiently oxidised to their corresponding sulphoxides at neutral pH in the presence of Mn^{2+}, oxygen and sulphite (Yang, 1970). The sulphoxide formation is dependent on the aerobic oxidation of sulphite. The available data are in good agreement with the mechanism depicted below in which OH· or O_2^- serves as the oxidising agent:

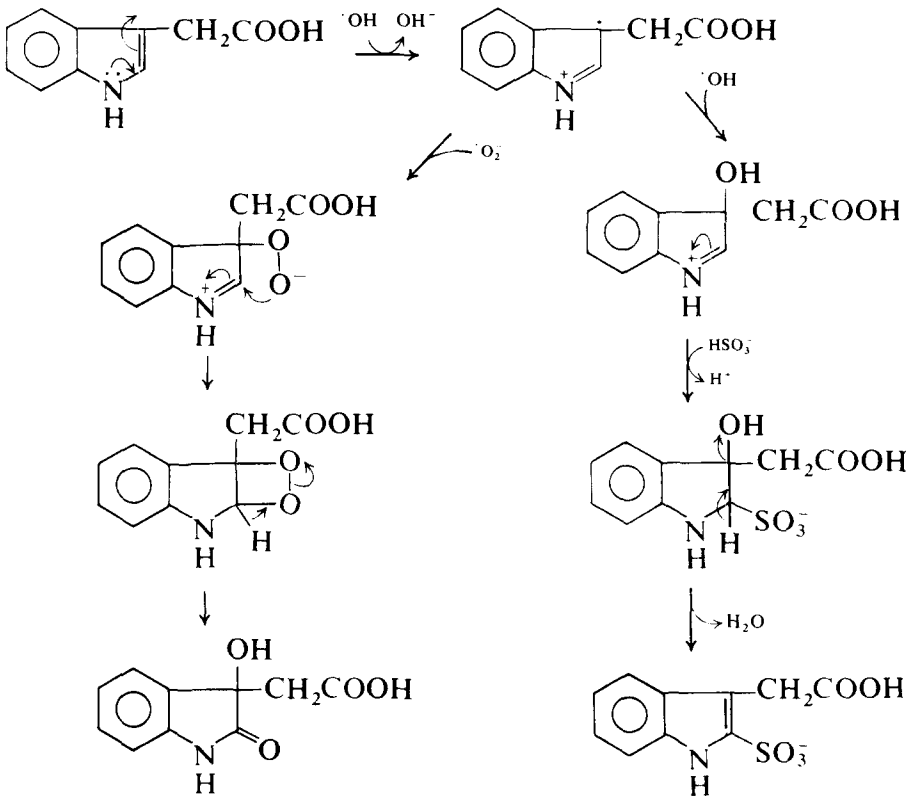


In addition to methionine, tryptophan is also readily oxidised during the aerobic oxidation of sulphite (Yang, 1973). The destruction of tryptophan was dependent on the aerobic oxidation of sulphite; superoxide dismutase inhibited sulphate oxidation and thereby the tryptophan destruction. Although the oxidation products of tryptophan were not identified, tracer studies indicated that trytophan was converted into at least four products.

OXIDATION OF INDOLE-3-ACETIC ACID

Analogous to the destruction of tryptophan, indole-3-acetic acid, an important plant hormone, is also rapidly destroyed when coupled to the oxidation of sulphate (Yang & Saleh, 1973). Tracer studies indicated that indole-3-acetic acid was converted into two compounds. Unlike the other degradation pathways of indoleacetic acid, decarboxylation of indoleacetic acid was not involved in this sulphite-mediated destruction.

These two products were identified as dioxindole-3-acetic acid and (2-sulfoindole)-3-acetic acid (Horng & Yang, 1975). A chemical mechanism accounting for the reactions is shown below:



DESTRUCTION OF CHLOROPHYLLS

Destruction of chlorophyll (Chl) is one of the main symptoms of SO_2 injury in plants. The possible participation of free radicals, produced during the aerobic oxidation of sulphite, in the destruction of Chl was examined in three *in vitro* systems. The first system consisted of light and O_2 in addition to sulphite. The action spectrum of Chl in this system matches its absorption spectrum, indicating that Chl functions as a photosensitiser. In the second system, Chl destruction in the presence of

sulphite occurred in the dark, but required Mn^{2+}, O_2 and glycine. Destruction of Chl in this system was much more rapid than in the light system, with approximately 70% of the destruction occurring in 2 s (Peiser & Yang, 1977). In both systems, Chl destruction was linked to bisulphite oxidation. The free radical scavengers, hydroquinone, butylated hydroxytoluene, tiron (1,2-dihydroxybenzene-3,5-disulphonic acid) and α-tocopherol, were effective in inhibiting the destruction of Chl in both systems. The singlet O_2 scavengers, 2,5-dimethylfuran and 1,3-diphenylisobenzofuran, were ineffective as inhibitors and β-carotene was only slightly effective when tested in the light system. The evidence suggests that Chl was destroyed by free radicals, which were produced during the aerobic oxidation of sulphite initiated either by photosensitised Chl or by Mn^{2+}. Since the reaction was carried out in ethanol solution, which is a potent scavenger of the HO· radical, the HO· radical could not be the species responsible for Chl destruction. Superoxide radical was probably responsible for the destruction, since the destruction was inhibited by tiron, a scavenger of superoxide radical.

$$\text{Chl} + 2H^+ + O_2^- \text{ (or } HSO_3) \longrightarrow \text{Chl}^+ + H_2O_2 \text{ (or } HSO_3^-)$$

$$O_2 + \text{Chl}^+ \longrightarrow \text{oxidation products}$$

In the third system, Chl is destroyed by sulphite and linoleic acid hydroperoxide (LOOH). Both sulphite and linoleic acid hydroperoxide were required for chlorophyll destruction and both were consumed in the reaction; however, there was no oxygen requirement, and hydrogen peroxide could not replace LOOH (Peiser & Yang, 1978). Linolenic acid hydroperoxide is, however, as effective as LOOH. The free radical scavengers, hydroquinone and α-tocopherol, were very effective inhibitors of chlorophyll destruction, but the singlet oxygen quenchers, β-carotene, 2,5-dimethylfuran and 1,3-diphenylisobenzofuran, were only slightly effective. Based on the present results and that of others, it is suggested that chlorophyll was destroyed via oxidation by the alkoxy or bisulphite radical which was produced during the decomposition of linoleic acid hydroperoxide by bisulphite.

In all three *in vitro* systems described above, the chlorophyll used was partially purified and contained both Chl *a* and Chl *b*. It was noted from the absorption spectra that Chl *a* was preferentially destroyed over Chl *b*. Such a preferential destruction of Chl *a* was widely observed in senescent leaf tissue and in tissue exposed to SO_2.

DESTRUCTION OF β-CAROTENE

Since β-carotene is an important component of the chloroplast pigments which were destroyed by exposure to SO_2, we have investigated the destruction of β-carotene by the bisulphite-induced free radical system. β-Carotene destruction in the presence of sulphite, as determined by the loss in absorbance at 454 nm, occurred in either of two *in vitro* systems (Peiser & Yang, 1979*b*). In the first system, requiring Mn^{2+}, O_2 and glycine, in addition to sulphite, over 90% of the β-carotene was destroyed in 15 s. Destruction was effectively inhibited by the free radical scavengers, α-tocopherol, tiron (1,2-dihydroxybenzene-3,5-disulfonic acid) and BHT (butylated hydroxytoluene). The concentrations of these scavengers required to cause 50% inhibition were 5 μM, 9 μM and 5 mM, respectively. In the second system, about 80% of the β-carotene was destroyed in 15 s in the presence of bisulphite and LOOH and both of these reactants were consumed. There was no O_2 requirement in this system. The free radical scavengers, α-tocopherol and BHT, effectively inhibited the destruction of β-carotene, but the singlet oxygen quencher, 2,5-dimethylfuran, was only slightly effective. LOOH can be substituted with linolenic acid hydroperoxide but not by hydrogen peroxide. The evidence indicates that, in these two systems, β-carotene was destroyed by free radicals. In the first system, superoxide radical and/or sulphite radical, formed during the aerobic oxidation of bisulphite, was presumably responsible for β-carotene destruction. The first step consists of a hydrogen abstraction from β-carotene by these radicals and results in the formation of β-carotene radical.

$$SO_3^{2-} + Mn^{2+} + O_2 \rightarrow O_2^- + SO_3^- + Mn^{2+}$$

$$\beta\text{-carotene} + O_2^- + H^+ \rightarrow \beta\text{-carotene}\cdot + H_2O_2$$

$$\beta\text{-carotene} + SO_3^- \rightarrow \beta\text{-carotene}\cdot + H^+ + SO_3^{2-}$$

$$\beta\text{-carotene}\cdot + O_2 \rightarrow \beta\text{-carotene-OO}\cdot$$

$$\beta\text{-carotene-OO}\cdot \rightarrow \text{oxidation products}$$

The reaction products have recently been partially characterised by Wedzicha & Lamikanra (1983) to be highly oxygenated. In the second

system, bisulphite caused the homolytic cleavage of linoleic acid hydroperoxide, producing the alkoxy radical and sulphite radical which are, in turn, involved in the destruction of β-carotene.

$$\text{LOOH} + \text{SO}_3^{2-} \rightarrow \text{LO}\cdot + \text{SO}_3^{-} + \text{OH}^-$$

$$\beta\text{-carotene} + \text{LO}\cdot \rightarrow \beta\text{-carotene}\cdot + \text{LOH}$$

$$\beta\text{-carotene} + \text{SO}_3^{-} \rightarrow \beta\text{-carotene}\cdot + \text{H}^+ + \text{SO}_3^{2-}$$

$$\text{LOOH} + \beta\text{-carotene}\cdot \rightarrow \text{oxidation products of } \beta\text{-carotene}$$

LIPID PEROXIDATION AND MEMBRANE DAMAGE

The oxygen-dependent addition of bisulphite to unsaturated fatty acid has long been recognised and evidence indicates that the reaction proceeds via a free radical mechanism (Gilbert, 1965). Kaplan *et al.* (1975) and Lizada & Yang (1981) have shown that linoleic acid and linolenic acid were oxidised in the presence of sulphite to form the corresponding hydroperoxides. The addition of sulphite to an emulsion of linoleic acid (LH) initiated the formation of LOOH. Peroxidation required oxygen and sulphite and occurred with concomitant oxidation of sulphite. The time course shows that the formation of peroxidised linoleic acid correlated with the disappearance of sulphite; the content of conjugated diene, as measured by absorbancy at 232 nm, equaled the peroxide content assayed by the ferrous thiocyanate reagent. To characterise the structure of LOOH, LOOH was first isolated by TLC and was converted into methyl hydroxystearate by successive reduction, methylation and hydrogenation. GC–MS analysis of the methyl hydroxystearate indicated that LOOH contained both the 9- and 13-hydroperoxide isomers. When the reaction was carried out with [^{14}C-U]LH and NaH^{35}SO$_3$, a bisulphite adduct was also detected. When linolenic acid was used in place of linoleic acid, hydroperoxide was detected, but the reaction proceeded at a much slower rate than that with linoleic acid. The free radical scavengers, hydroquinone and α-tocopherol, effectively inhibited both bisulphite oxidation and LH peroxidation. 4-Thiouridine blocked peroxidation, suggesting that SO_3^- radical is an essential intermediate. Neither superoxide dismutase, catalase nor hydroxyl radical scavengers (mannitol or *t*-butanol) significantly inhibited the reaction. Mn^{2+}, however, enhanced bisulphite oxidation, but inhibited LH peroxidation.

The following reactions are suggested to account for the sulphite-mediated lipid peroxidation.

Initiation:

$$SO_3^{2-} + O_2 \rightarrow SO_3^- + O_2^-$$

Propagation:

$$LH + SO_3^- \rightarrow L\cdot + H^+ + SO_3^{2-}$$
$$L\cdot + O_2 \rightarrow LOO\cdot$$
$$LOO\cdot + LH \rightarrow LOOH + L\cdot$$
$$LOO\cdot + H^+ + SO_3^{2-} \rightarrow LOOH + SO_3^-$$

Although the primary cause of SO_2 injury to plants is not clear, membrane damage has been implicated, based on the observation that swelling of thylakoids in chloroplasts is the first ultrastructural change occurring in the SO_2-exposed leaves (Wellburn *et al.*, 1972). Ethane formation from tissues has been employed as a measure for lipid peroxidation (Riely *et al.*, 1974; Dillard & Tappel, 1979). Ethane production from plants exposed to SO_2 has been reported (Peiser & Yang, 1979*a*, Bressan *et al.*, 1979). Recently, we have examined the effect of sulphite on the peroxidation of isolated chloroplast membranes (Peiser *et al.*, 1982). Isolated spinach chloroplasts were used in this study and ethane evolution was used as a measure of lipid peroxidation. In broken and intact chloroplasts, sulphite greatly stimulated ethane formation compared with the sulphate controls. As observed in other systems involving the free-radical oxidation of sulphite, ethane formation depends on sulphite oxidation. Light was required for both ethane formation and sulphite oxidation. Earlier, Asada & Kiso (1973) studied the oxidation of sulphite by illuminated chloroplasts and observed that photosynthetic electron transport was involved. They concluded that the free-radical oxidation of sulphite was initiated by superoxide radical which was formed on the reducing side of photosystem I (one of two functional light-harvesting systems in chloroplast during photosynthesis). In our system, we observed that photosynthetic electron transport was required for both sulphite oxidation and ethane formation. DCMU [3(3,4-dichlorophenyl)-1,1-dimethylurea], which is an inhibitor of photosynthetic electron transport, and phenazine methosulphate (PMS), which promotes cyclic electron flow, inhibited both sulphite oxidation and ethane formation. The free radical scavengers, tiron and ascorbate, effectively

inhibited both sulphite oxidation and ethane formation. The specific involvement of superoxide radical was implicated since superoxide dismutase inhibited ethane formation. Photosynthetic electron transport provides electrons to reduce O_2 to O_2^-, which initiates sulphite oxidation. Radicals produced from sulphite oxidation then lead to the peroxidation of membrane lipids, resulting in the formation of ethane. These results show that free radicals generated from the aerobic oxidation of sulphite can cause damage to isolated chloroplasts.

CONCLUDING REMARKS

Sulphite can react with organic compounds in the biological material via sulphonation reactions or can undergo aerobic oxidation to sulphate with the concomitant formation of free radicals, which may, in turn, actively react with many cellular components. Although sulphonation reactions primarily occur by an ionic mechanism at high concentrations of sulphite, sulphite undergoes free-radical oxidation at low concentrations (Inoue & Hayatsu, 1971). The investigations summarised above indicate that sulphate can cause oxidation of a number of biologically important molecules *in vitro*. However, it has yet to be determined to what extent such free radical reactions occur in plant tissues.

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