Sulfoxide Formation from Methionine or Its Sulfide Analogs during Aerobic Oxidation of Sulfite*

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ABSTRACT: Methionine and other sulfides were rapidly and efficiently oxidized to their corresponding sulfoxides at neutral pH in the presence of Mn^{2+}, oxygen, and sulfite ion. The sulfoxide formation was dependent on the aerobic oxidation of sulfite. In the presence of excess methionine, more than 0.5 mole of methionine was oxidized to sulfoxide per mole of sulfite oxidized. Superoxide dismutase inhibited sulfite oxidation and thus inhibited sulfoxide formation. Other sulfides, such as *S*-methylcysteine, were not appreciably oxidized by the Mn–sulfite–O_2 system, but were rapidly oxidized when horseradish peroxidase, Mn^{2+}, and phenol were included.

The peroxidase-catalyzed sulfoxide formation was not inhibited by superoxide dismutase. A chemical mechanism accounting for the sulfoxide formation by the present systems is presented in which superoxide anion and hydroxy radicals produced during the aerobic oxidation of sulfite function as oxidizing agents.

During the study of ethylene production from 4-methylthio-2-oxobutanoic acid catalyzed by horseradish peroxidase (HRP)[1] in the presence of sulfite and oxygen, 3-(methylsulfinyl)propionic acid was identified as a major by-product (Yang, 1969). The data suggested that the sulfide function may be readily oxidized to sulfoxide during the reaction. Methionine is known to be oxidized to methionine sulfoxide by various oxidants (Greenstein and Winitz, 1961), including hydrogen peroxide in acidic medium (Iselin, 1961), or by dye-sensitized photooxidation (Weil *et al.*, 1951; Strauss and Nickerson, 1961; Jori *et al.*, 1968). The present paper shows that methionine and its sulfide analogs are rapidly and efficiently oxidized to their corresponding sulfoxides at neutral pH during the aerobic oxidation of sulfite ion. Under similar reaction conditions, methionine was not appreciably oxidized by hydrogen peroxide. A chemical mechanism is presented to account for the formation of sulfoxide and for the catalytic role of peroxidase in the reaction.

Experimental Section

Materials. L-Methionylglycine was obtained from the Cyclo Chemical Co. The other amino acids were obtained from Calbiochem. L-[*methyl*-^{14}C]Methionine was purchased from New England Nuclear. 4-Methylthio-2-oxobutanoic acid was prepared enzymatically as described previously (Yang, 1969). 3-Methylthiopropionic acid was prepared by the method of Hurd and Gershbein (1947); 3-methylthiopropylamine was prepared from methionine with acetophenone (Obata and Ishikawa, 1966). Horseradish peroxidase, Type II, was purchased from Sigma. Pure superoxide dismutase from bovine erythrocytes (McCord and Fridovich, 1969a) was kindly provided by Dr. I. Fridovich. Sodium bisulfite and hydrogen peroxide were Baker reagent grade. All other materials were commercial products.

Methods. The reactions were conducted in 13 × 10 mm Hycel cuvet which are used with the Bausch & Lomb Spectronic 20 spectrophotometer. A typical incubation mixture contained, in a total volume of 0.2 ml, 0.2 μmole of methionine or other sulfides, 10 μmoles of phosphate buffer at pH 6.8, 0.5 μmole of sodium bisulfite, and 10 μmoles of $MnSO_4$. The reaction was started by the addition of bisulfite. The reaction proceeded at 25° and was terminated by the addition of 0.05 ml of 2 M formaldehyde. After 5 min, 4 ml of iodoplatinate reagent (Awwad and Adelstein, 1966) was added to the reaction tubes and the sulfide content remaining in the reaction mixture was determined colorimetrically with a Spectronic 20 spectrophotometer. The concentration of iodoplatinate reagent was so adjusted that the optical density of the reagent itself was about 0.75 and the decrease in optical density in the presence of 0.1 μmole of methionine was about 0.3. Bleaching by sulfoxide has been reported to be negligible (Sease *et al.*, 1948), and it does not interfere with the assay. The decrease in sulfide content during the reaction was taken as equivalent to sulfoxide formation.

The uptake of oxygen was measured with an oxygen electrode (Yang, 1967). The concentrations of the reaction components were the same as listed above but with a total volume of 3 ml.

Product identification was conducted by paper or silica gel thin-layer chromatography developed with 1-butanol–acetic acid–water (4:1:5, v/v).

Results

Identification of Reaction Products of Methionine. For the identification of the reaction products, the reaction mixture containing methionine as substrate was incubated for 30 min and chromatographed on paper and on silica gel thin-layer plates using 1-butanol–acetic acid–water (4:1:5, v/v) as the developing solvent. Spraying with ninhydrin reagent revealed two spots which had R_F values corresponding, respectively,

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[1] The abbreviation used is: HRP, horseradish peroxidase.

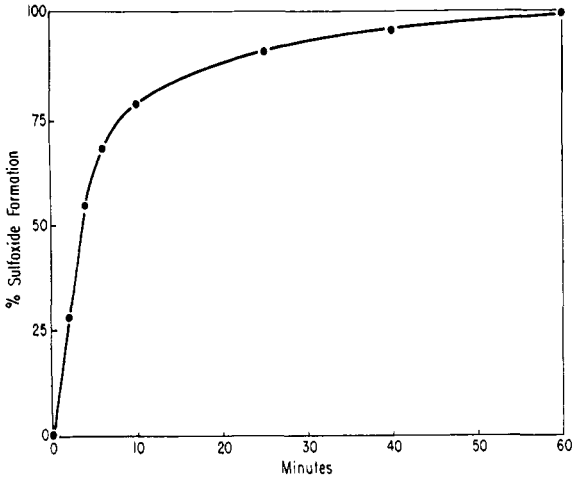

FIGURE 1: Time course of methionine sulfoxide formation from methionine. The reaction mixture contained, in a total volume of 0.2 ml, 0.2 μmole of methionine, 10 μmoles of phosphate buffer at pH 6.8, 0.5 μmole of sodium bisulfite, and 10 mμmole of $MnSO_4$. Incubation was at 25°.

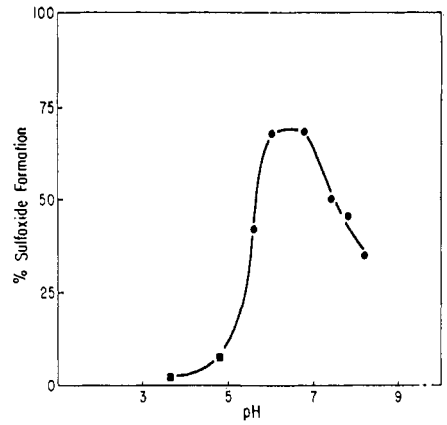

FIGURE 2: Dependence of methionine sulfoxide formation on pH. Reaction mixture and conditions were as described in Figure 1, except that the incubation time was 10 min and 10 μmoles of either phosphate (●) or acetate (■) buffer was employed.

to methionine and to methionine sulfoxide. Additional evidence that the product was methionine sulfoxide was derived from the observation that it was reduced to methionine when it was reacted with mercaptoethanol (Jori *et al.*, 1968). If methionine sulfone were the product, it would not be reduced by mercaptoethanol. In order to determine whether other products might be formed but not be detected by the ninhydrin reaction, 4×10^5 dpm of L-[*methyl*-^{14}C]methionine was included in the reaction mixture. After a 30-min reaction period, an aliquot was chromatographed on paper and the chromatogram scanned for radioactivity. The radiochromatogram indicated that 85% of the total radioactivity was recovered as methionine sulfoxide (R_F 0.22) and 12% as methionine (R_F 0.50). The rest of the radioactivity (3%) was found in two unknown spots with R_F values of 0.73 and 0.33. Further identification of the two minor components was not attempted. When the oxidized reaction mixture was reacted at 100° for 1 hr in 10% of mercaptoethanol and then chromatographed, 91% of the total radioactivity was recovered as methionine and the remaining 9% as methionine sulfoxide. These results provide strong evidence that the major product was methionine sulfoxide.

Characteristics of Methionine Sulfoxide Formation from Methionine. The time curve for conversion of methionine into methionine sulfoxide under standard conditions is shown in Figure 1. Methionine conversion was proportional to time only during the first 4 min. More than 75% of methionine was oxidized during 10-min incubation and the conversion was essentially completed by the end of 1 hr. The optimal pH for the system was between 6 and 7 (Figure 2). Table I illustrates the requirements for Mn^{2+}, SO_3^{2-}, and oxygen; when any one of them was omitted, there was little or no formation of sulfoxide. Hydrogen peroxide has been widely used as an oxidant to oxidize methionine to methionine sulfoxide (Greenstein and Winitz, 1961; Iselin, 1961). When 0.5 or 2 μmoles of H_2O_2 was substituted for 0.5 μmole of sulfite in this system, no appreciable oxidation of methionine was observed. It is indicated that Mn^{2+}–sulfite–oxygen constitutes a far more efficient system than H_2O_2 for the oxidation of methionine under these conditions.

The dependence of methionine oxidation on the concentration of Mn^{2+} and SO_3^{2-} is recorded in Table II. The reaction rate increased progressively as the amount of Mn^{2+} was increased from 0 to 100 mμmoles. Though there was no apparent optimal concentration, the rate of increase lessened when the amount of Mn^{2+} exceeded 3 mμmoles. Under the present reaction conditions with a reaction time of 5 min, the optimal concentration of sulfite ion for maximum rate of sulfoxide formation was found to be 1 μmole in 0.2 ml of reaction mixture (Table II). The oxidation of methionine in this system is dependent on the availability of sulfite. In reaction mixtures containing little sulfite (20 or 50 mμmoles), the oxidation of methionine essentially stopped after 5 min,

TABLE I: Methionine Sulfoxide Formation from Methionine by the Mn^{2+}–SO_3^{2-}–O_2 System and by Hydrogen Peroxide.

Components	Sulfoxide Formation (%)[c]
Complete[a]	75
$-Mn^{2+}$	4
$-SO_3^{2-}$	0
$-Mn^{2+}$, $-SO_3^{2-}$, +0.5 μmole of H_2O_2	1
$-Mn^{2+}$, $-SO_3^{2-}$, +2 μmoles of H_2O_2	3
Complete,[b] bubbled with air	80
Complete,[b] bubbled with N_2	2

[a] The complete reaction mixture contained, in a total volume of 0.2 ml, 0.2 μmole of methionine, 10 μmoles of phosphate at pH 6.8, 0.5 μmole of $NaHSO_3$, and 10 mμmoles of $MnSO_4$. Incubation was at 25° for 10 min. [b] The reaction mixture contained, in a total volume of 1 ml, 1 μmole of methionine, 2 μmoles of $NaHSO_3$, 50 μmoles of phosphate at pH 6.8, and 50 mμmmoles of $MnSO_4$. Incubation was at 25 for 10 min. [c] Sulfoxide formation was expressed as per cent of methionine.

TABLE II: Dependence of Methionine Oxidation on the Concentration of Mn^{2+} and Sulfite.[a]

	Concn (mμmoles)	Sulfoxide Formation (%) 5 min	60 min
$MnSO_4$	0	0	
	1	6	
	3	44	
	10	51	
	30	58	
	100	64	
$NaHSO_3$	0	0	0
	20	6	6
	50	11	14
	100	19	26
	200	29	53
	500	44	95
	1000	49	97
	2000	45	98

[a] The reaction mixtures and conditions were as those described in Figure 1, except that the concentration of $MnSO_4$ and $NaHSO_3$ and the incubation times were varied as indicated.

TABLE III: Effects of Mannitol, Superoxide Dismutase, and Horseradish Peroxidase on the Oxidation of Methionine.[a]

Addition	Sulfoxide Formation (%)
Experiment 1	
None	75
+0.5 μmole of mannitol	67
+5 μmoles of mannitol	55
+25 μmoles of mannitol	14
+25 μmoles of mannitol + HRP	38
+25 μmoles of mannitol + phenol	11
+25 μmoles of mannitol + HRP + phenol	78
Experiment 2	
None	80
+0.1 μg of dismutase	59
+0.2 μg of dismutase	40
+0.5 μg of dismutase	8
+0.5 μg of dismutase + phenol + HRP	79

[a] The reaction mixture and conditions were as in Figure 1, except that various amounts of mannitol or superoxide dismutase, 10 mμmoles of phenol, or 1 μg of HRP was added where indicated. Incubation time was 10 min.

presumably because all of the sulfite had been oxidized. When additional sulfite was added, oxidation of methionine resumed. In order to study the quantitative relationship between the amount of sulfite provided and the amount of sulfoxide found, the reaction time was prolonged to 1 hr, so that reaction time would not become a limiting factor. In the reaction mixture containing sulfite in smaller concentration than, or equal to, that of methionine (200 mμmoles) (so that methionine would not become limiting), greater than 0.5 mole of methionine sulfoxide was formed per mole of sulfite provided. In those reaction mixtures containing sulfite in larger concentration than that of methionine, the oxidation efficiency in term of sulfite required was poor, because essentially all of the methionine present had been oxidized to sulfoxide (Table II).

HRP is known to catalyze the aerobic oxidation of sulfite and reactions coupled to this oxidation (Fridovich and Handler, 1961; Klebanoff, 1961; Yang, 1967, 1969). The reaction rate of the present Mn^{2+}–sulfite–oxygen system, however, was so fast that no catalytical effect of HRP was detected. Mannitol (Fuller and Crist, 1941) and superoxide dismutase (McCord and Fridovich, 1969b) have been shown to effectively inhibit the aerobic oxidation of sulfite ion. When the Mn^{2+}–sulfite–oxygen system was inhibited by mannitol, the catalytic effect of peroxidase on the oxidation of methionine was readily demonstrated (Table III). In the presence of 25 μmoles of mannitol, sulfoxide formation was inhibited by more than 80%, but when peroxidase and phenol were included the inhibition by mannitol was completely abolished. Table III also illustrates that phenol is required for maximum peroxidase-catalyzed sulfoxide formation. The requirements for Mn^{2+} and monophenol (or *m*-dihydric phenol) as cofactors in various oxidatic reactions catalyzed by peroxidase have long been recognized (Goldacre *et al.*, 1953; Akazawa and Conn, 1958; Fridovich and Handler, 1961; Kay *et al.*, 1967; Yang, 1967). Superoxide dismutase was found to be a very potent inhibitor of sulfoxide formation. In the presence of 0.2 μg of superoxide dismutase, the rate of methionine sulfoxide formation was inhibited 50%, while in the presence of 0.5 μg the inhibition was about 90% (Table III). The inhibition was completely abolished when phenol and HRP were added. Thus, dismutase and mannitol inhibited sulfoxide formation in the Mn^{2+}-catalyzed nonenzymic system but not in the peroxidase-catalyzed system, indicating that the mechanism responsible for the oxidation of methionine in the nonenzymic system is different from that in the peroxidase system.

Sulfoxide Formation from the Other Dialkyl Sulfides. In order to ascertain whether the present system works on sulfide compounds other than methionine, some methionine-related compounds were tested for sulfoxide formation by the Mn^{2+}–sulfite–oxygen system. The results are shown in Table IV. Substitution of an ethyl group (ethionine) for the methyl group of methionine did not affect the efficiency of sulfoxide formation. The carboxyl group of methionine could be either substituted (methionylglycine) or eliminated (3-methylthiopropylamine) without significant change in efficiency. However when the amino group of methionine was either replaced by a keto group (4-methylthio-2-oxobutanoic acid) or acylated (*N*-acetylmethionine), there was little or no sulfoxide formation. It appears, therefore, that a free amino group is essential as an active substrate. It is interesting that *S*-methylcysteine, a methionine homolog was only slightly active, as was 3-methylthiopropionic acid. Table V shows the

TABLE IV: Sulfoxide Formation from Various Methionine Analogs.[a]

Substrate	Sulfoxide Formation (%)
Methionine	80
Ethionine	79
Methionylglycine	65
3-Methylthiopropylamine	80
4-Methylthio-2-oxobutanoic acid	0
N-Acetylmethionine	8
S-Methylcysteine	2
3-Methylthiopropionic acid	8

[a] The reaction mixtures and conditions were as those described in Figure 1, except that 0.2 μmole of various sulfides was substituted for methionine. Incubation time was 10 min.

TABLE V: Stimulation of Sulfoxide Formation from Various Sulfides by Horseradish Peroxidase or by High Concentration of Mn^{2+} Ion.[a]

Substrate	Addition	Sulfoxide Formation (%)
N-Acetylmethionine	None	0
N-Acetylmethionine	Mn^{2+} (10 mμmoles)	8
N-Acetylmethionine	Mn^{2+} (10 mμmoles) + phenol + HRP	43
N-Acetylmethionine	Mn^{2+} (100 mμmoles)	48
N-Acetylmethionine	Mn^{2+} (500 mμmoles)	67
S-Methylcysteine	None	0
S-Methylcysteine	Mn^{2+} (10 mμmoles)	4
S-Methylcysteine	Mn^{2+} (10 mμmoles) + phenol + HRP	50
S-Methylcysteine	Mn^{2+} (100 mμmoles)	10
S-Methylcysteine	Mn^{2+} (500 mμmoles)	12
3-Methylthiopropionic acid	None	0
3-Methylthiopropionic acid	Mn^{2+} (10 mμmoles)	9
3-Methylthiopropionic acid	Mn^{2+} (10 mμmoles) + phenol + HRP	45
3-Methylthiopropionic acid	Mn^{2+} (100 mμmoles)	30
3-Methylthiopropionic acid	Mn^{2+} (500 mμmoles)	50

[a] The standard reaction mixture and conditions were as those described in Figure 1, except that various additions as indicated and 0.2 μmole of various substrates were substituted for Mn^{2+} and methionine. Incubation time was 10 min. The amount of phenol added is 10 mμmoles, HRP, 1 μg.

stimulation of sulfoxide formation from *N*-acetylmethionine, *S*-methylcysteine, and 3-methylthiopropionic acid by peroxidase or by higher concentrations of Mn^{2+}. These sulfides were only slightly oxidized under the standard conditions (Table IV) but with the addition of 1 μg of HRP and 10 mμmoles of phenol the oxidation rate increased 5–10 times. A high concentration of Mn^{2+} was also found to be effective in stimulating the oxidation of *N*-acetylmethionine and 3-methylthiopropionic acid, but only slightly effective toward *S*-methylcysteine.

Relationship between Sulfoxide Formation and Sulfite Oxidation. In order to elucidate the relationship between sulfoxide formation and sulfite oxidation, oxygen uptake was measured in reaction mixtures of essentially the same composition as those used for assay of sulfoxide formation. When sulfide was omitted from the reaction mixtures, the system was found to retain the ability to take up oxygen rapidly, but if sulfite was omitted there was no oxygen uptake. The results indicate that the oxidation of sulfide is initiated by the oxidation of sulfite, but the oxidation of sulfite does not require the oxidation of sulfide. The initiation of oxygen uptake, as shown in Figure 3, must therefore represent the initiation of sulfite oxidation, though the total oxygen uptake represents oxidation of both sulfite and sulfide. The ability of metal ions to initiate the aerobic oxidation of sulfite to sulfate is well known (Fuller and Crist, 1941; Abel, 1951; Fridovich and Handler, 1961; Yang, 1967). In the present system all agents which induce sulfoxide formation also induce sulfite oxidation, and agents which inhibit sulfoxide formation also inhibit sulfite oxidation. Figure 3A shows the initiation of sulfite oxidation by Mn^{2+}. In the presence of 1 or 2 μg of superoxide dismutase in 3 ml of reaction mixture, not only was the rate of oxygen uptake inhibited but also there was a lag period preceding the steady state of oxygen uptake (Figure 3B,C). The lag period was 1 or 2 min in the presence of 1 or 2 μg of superoxide dismutase, respectively. In the presence of 5 μg of superoxide dismutase, no increase in oxygen uptake was observed even at 10 min after the addition of Mn^{2+}. However, when HRP–phenol was added, an immediate increase in oxygen uptake was observed (Figure 3D). The inhibition of the Mn^{2+}-initiated sulfite oxidation by superoxide dismutase, and the counteraction by HRP–phenol, were analogous to the effects on sulfoxide formation (Table III). In the presence of *N*-acetylmethionine the rate of oxygen uptake was greatly stimulated by the addition of 5 μmoles/3 ml of Mn^{2+} or a mixture of HRP and phenol but not by a lower concentration (0.15 μmole) of Mn^{2+} (Figure 3E–G). Similar results were obtained in the presence of 3-methylthiopropionic acid (Figure 3H,I). In the presence of *S*-methylcysteine, the rate of oxygen uptake was not significantly accelerated by 5 μmoles of Mn^{2+}, but was greatly stimulated by peroxidase–phenol mixture (Figure 3J). These data indicate that the initiation of sulfite oxidation and the sulfoxide formation (Table V) are closely interrelated.

Discussion

The existence of a free-radical chain mechanism for the aerobic oxidation of sulfite to sulfate has been well documented (Abel, 1951). Sulfite oxidation can be initiated by

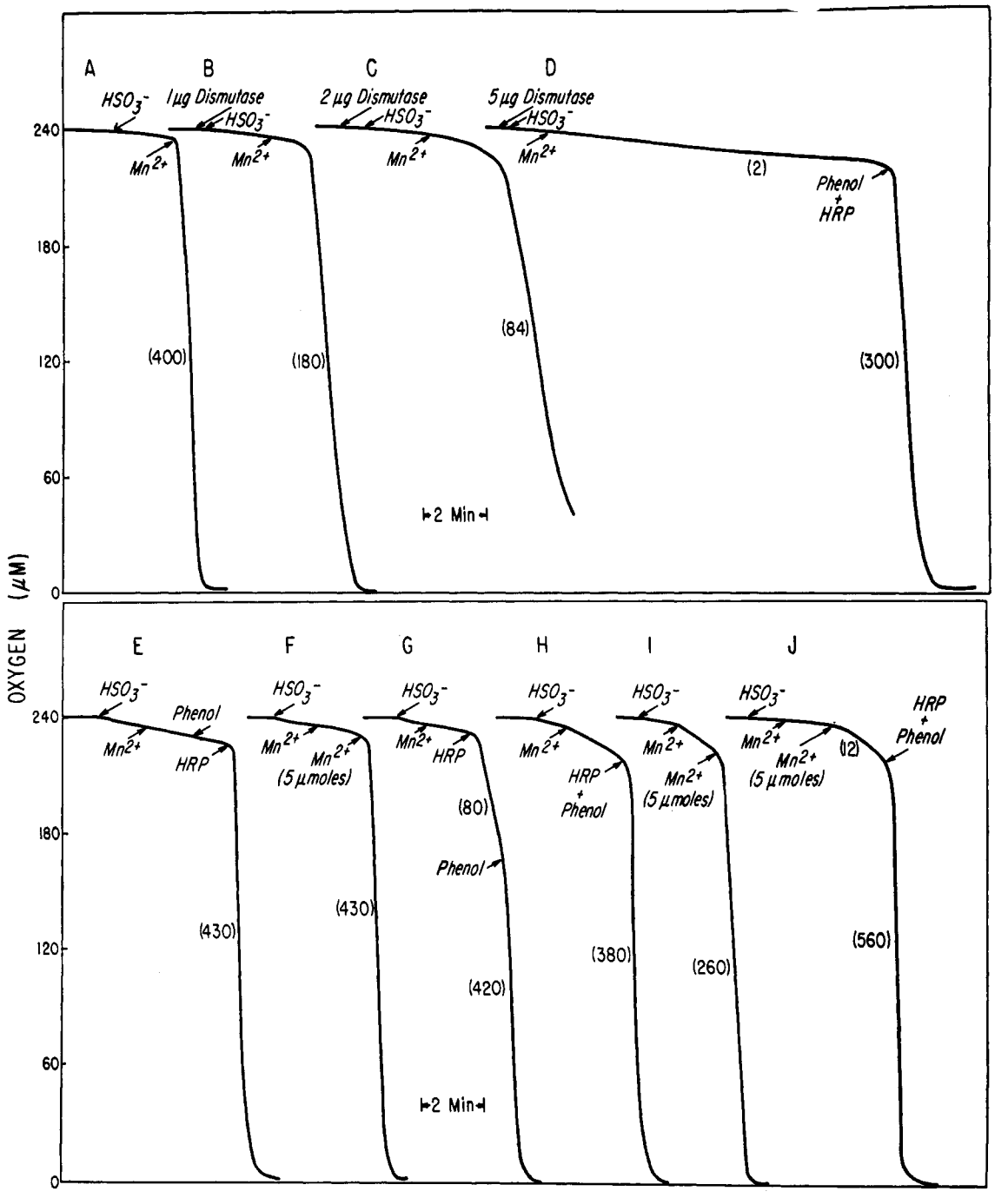

FIGURE 3: Oxygen uptake by the Mn^{2+}–sulfite–oxygen system in the presence of various sulfides. Oxygen uptake was measured with an oxygen electrode which was immersed in 2.8 ml of reaction mixture containing 3 μmoles of methionine or one of its analogs and 120 μmoles of phosphate buffer at pH 6.8. Various components in 0.05 ml of 50 μM phosphate buffer at pH 6.8 were then added as indicated. The amounts of the additions were 7.5 μmoles of $NaHSO_3$, 0.15 μmole, or 5 μmoles of $MnSO_4$ as indicated, 0.15 μmole of phenol, 15 μg of HRP, and various amounts of superoxide dismutase as indicated. A, B, C, and D contained methionine; E, F, and G contained *N*-acetylmethionine, H and I contained 3-methylthiopropionic acid, and J contained *S*-methylcysteine. The numbers in parantheses represent the rate of oxygen uptake in μM of oxygen/min.

metal ions (Abel, 1951) or by photochemical (Fridovich and Handler, 1960b), electrolytic (Fridovich and Handler, 1961) or enzymic (Fridovich and Handler, 1958, 1961; Klebanoff, 1961; Yip and Hadley, 1966; Yang, 1967) processes which are capable of generating free radicals. The early work of Fridovich and Handler (1958, 1960a, 1961) has strongly suggested the involvement of superoxide anion radical, O_2^-, in the induction of sulfite oxidation. Recently McCord and Fridovich (1969a,b) further demonstrated that erythrocuprein, a potent superoxide dismutase which catalyzes the disproportionation of superoxide radical anion (eq 1) strongly inhibits the spontaneous oxidation of sulfite in the presence of EDTA.

$$O_2^- + O_2^- + 2H^+ \longrightarrow O_2 + H_2O_2 \quad (1)$$

Indeed, the formation of O_2^- radical in the aerobic oxidation of dithionite has been recently demonstrated by Knowles *et al.* (1969) by means of electron paramagnetic resonance technique.

The initiation of sulfite oxidation by horseradish peroxidase in the presence of H_2O_2 and phenol (Fridovich and Handler, 1961) or in the presence of Mn^{2+} and a phenolic compound (Klebanoff, 1961; Yang, 1967) has been reported. The generation of superoxide radical in the peroxidase-catalyzed oxidase reaction has also been proposed by Yamazaki and Piette (1963).

Based on this information, the formation of initiating radicals in the Mn^{2+}-catalyzed sulfite oxidation may be expressed by eq 2 and 3 (Abel, 1951).

$$Mn^{2+} + O_2 \longrightarrow Mn^{3+} + O_2^- \quad (2)$$

$$Mn^{3+} + SO_3^{2-} + H^+ \longrightarrow Mn^{2+} + HSO_3 \quad (3)$$

Similarly, eq 4 and 5 may be written to represent the formation of the initiating radicals O_2^- and HSO_3 by the HRP–phenol (ArOH) system. The formation of phenoxy radical (ArO) in the peroxidase-catalyzed reactions has been confirmed (Caldwell and Steelink, 1969).

$$ArOH + O_2 \xrightarrow{HRP\text{-}Mn^{2+}} ArO + O_2^- + H^+ \quad (4)$$

$$ArO + SO_3^{2-} + 2H^+ \longrightarrow ArOH + HSO_3 \quad (5)$$

Once the chain-initiating species O_2^- and HSO_3 are formed, sulfite oxidation is maintained through the chain-propagating reactions (eq 6, 7, and 8), with the concomitant formation of sulfate (eq 8, 9, 10, and 11)

$$SO_3^{2-} + O_2^- + 3H^+ \longrightarrow HSO_3 + 2OH \quad (6)$$

$$SO_3^{2-} + OH + 2H^+ \longrightarrow HSO_3 + H_2O \quad (7)$$

$$HSO_3 + O_2 \longrightarrow SO_3 + O_2^- + H^+ \quad (8)$$

$$HSO_3 + OH \longrightarrow SO_3 + H_2O \quad (9)$$

$$2HSO_3 \longrightarrow SO_3 + SO_3^{2-} + 2H^+ \quad (10)$$

$$SO_3 + H_2O \longrightarrow SO_4^{2-} + 2H^+ \quad (11)$$

Although both O_2^- and HSO_3 are able to initiate the chain-propagating reactions, maintenance of the chain propagations initiated by HSO_3 radical would depend on the secondary formation of O_2^- radical as represented by eq 8. Therefore it is expected that superoxide dismutase would inhibit both O_2^-- and HSO_3-initiated chain propagation. The results of Figure 3 indicate that superoxide dismutase inhibited Mn^{2+}-mediated sulfite oxidation but did not inhibit HRP-mediated sulfite oxidation. This may be explained by the assumption that O_2^- radical generated by peroxidase was so tightly bound to enzyme (Yamazaki and Piette, 1963) and was effectively reduced by the sulfite (eq 6) that superoxide dismutase did not effectively scavenge the O_2^- radical. In this regard, it is pertinent to note the observation of Massey *et al.* (1969) that superoxide dismutase was ineffective as an inhibitor of several flavoprotein-catalyzed oxidations. Alternately, the inactivity of superoxide dismutase to inhibit peroxidase-mediated sulfite oxidation may be explained on the basis that this oxidation is initiated by agents other than O_2^-.

The present results have shown that sulfoxide formation is initiated by the aerobic oxidation of sulfite, and that sulfoxide formation and sulfite oxidation are inhibited by the same inhibitors and initiated by the same activators. Since neither sulfite alone nor sulfate could initiate the oxidation of sulfide, it must be assumed that one or more of those agents which are produced during the sulfite–oxygen chain reaction then react with the sulfides to form sulfoxides. There is considerable evidence that the first step in the reaction of a sulfide with a one-electron oxidant is the transfer of an electron from the sulfur atom of sulfide to yield sulfonium radical as shown by eq 12 (Yang, 1967, 1969; Yang *et al.*, 1967). Subsequent reaction of this dialkylsulfonium radical with OH radical generated during the sulfite–oxygen chain reaction should readily yield sulfoxide (eq 13).

$$R{-}S{-}R' + O_2^- + 2H^+ \text{ (or OH)} \longrightarrow R{-}\overset{+}{S}{-}R' + 2OH \text{ (or } OH^-) \quad (12)$$

$$R{-}\overset{+}{S}{-}R' + OH \longrightarrow R{-}\overset{+}{S}(-O{-}H){-}R' \longrightarrow R{-}\overset{+}{S}(=O){-}R' + H^+ \quad (13)$$

Alternately the sulfonium radical may react with O_2^- radical to form an unstable persulfoxide (eq 14), which would oxidize in turn a second mole of sulfide to form 2 moles of sulfoxide (eq 15). This scheme is similar to that described by Foote (1968) for the methionine oxidation to methionine sulfoxide by singlet oxygen.

$$R{-}\overset{+}{S}{-}R' + O_2^- \longrightarrow RR'{-}\overset{+}{S}{-}O{-}O^- \quad (14)$$

$$RR'{-}\overset{+}{S}{-}O{-}O^- + R{-}S{-}R' \longrightarrow 2R{-}\overset{+}{S}(=O){-}R' \quad (15)$$

Since 2 moles of sulfite is required for the oxidation of 1 mole of methionine, the overall reaction of methionine sulfoxide formation may be written by eq 16 where R stands for $CH_2CH_2CHNH_2COOH$ group.

$$CH_3{-}S{-}R + 2SO_3^{2-} + 1.5\,O_2 \longrightarrow CH_3{-}\overset{+}{S}(=O){-}R + 2SO_4^{2-} \quad (16)$$

Methionine sulfoxide can also be formed efficiently from methionine by dye-sensitized photooxidation (Weil *et al.*, 1951; Strauss and Nickerson, 1961; Jori *et al.*, 1968). Since the sulfite–oxygen chain reaction and the dye-sensitized photo reactions are similar and may share the common intermediates (Fridovich and Handler, 1960b; Yang *et al.*, 1967; Ballou *et al.*, 1969), the mechanism described above may also be applicable to the dye-sensitized photooxidation. It is further suggested that such free-radical mechanism may

be responsible for the biological formation of sulfoxide *in vivo*.

Methionine, ethionine, methionylglycine, and 3-methylthiopropylamine are readily oxidized in the presence of sulfite and low concentration of Mn^{2+}, while *N*-acetylmethionine, *S*-methylcysteine, and 3-methylthiopropionic acid are not (Table IV). The inactivity of the latter sulfides to oxidation was due to the inhibition of the sulfite oxidation (Figure 3). Since a higher concentration of Mn^{2+} was able to relieve the inhibition (Table IV and Figure 3), it would appear possible that Mn^{2+} may form a complex with either *N*-acetylmethionine, *S*-methylcysteine, or 3-methylthiopropionic acid, so that the concentration of Mn^{2+} available to initiate sulfite oxidation was greatly decreased. However, *S*-methylcysteine has been shown not to complex with Mn^{2+} (McCormick *et al.*, 1969). A simple explanation is that the latter sulfides are able to scavenge the radicals which propagate the sulfite–oxygen chain reaction.

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