Volume 303, number 2,3, 213–216 FEBS 11089 June 1992

Xanthine oxidase/hydrogen peroxide generates sulfur trioxide anion radical ($SO_3^{\bullet-}$) from sulfite (SO_3^{2-})

Xiaoya Sun, Xianglin Shi and N.S. Dalal

Department of Chemistry, West Virginia University, Morgantown, WV 26506, USA

Received 27 March 1992

In the presence of hydrogen peroxide (H_2O_2), xanthine oxidase has been found to catalyze sulfur trioxide anion radical ($SO_3^{\bullet-}$) formation from sulfite anion (SO_3^{2-}). The $SO_3^{\bullet-}$ radical was identified by ESR (electron spin resonance) spin trapping, utilizing 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) as the spin trap. Inactivated xanthine oxidase does not catalyze $SO_3^{\bullet-}$ radical formation, implying a specific role for this enzyme. The initial rate of $SO_3^{\bullet-}$ radical formation increases linearly with xanthine oxidase concentration. Together, these observations indicate that the $SO_3^{\bullet-}$ generation occurs enzymatically. These results suggest a new property of xanthine oxidase and perhaps also a significant step in the mechanism of sulfite toxicity in cellular systems.

Xanthine oxidase; ESR; Spintrapping; Sulfite toxicity; Sulfur trioxide anion radical; Free radical

1. INTRODUCTION

During our recent studies of the generation of the sulfur trioxide anion radical ($SO_3^{\bullet-}$) from sulfite anion (SO_3^{2-}), we observed a new property of xanthine oxidase: in the presence of hydrogen peroxide (H_2O_2), this enzyme efficiently catalyzes the generation of $SO_3^{\bullet-}$ radical from SO_3^{2-}. The purpose of this communication is to document the evidence leading to this conclusion. The significance of this undertaking is as follows. First, xanthine oxidase, with xanthine as a substrate, is commonly utilized as a source of $O_2^{\bullet-}$ radical in examining the role of oxygenated radicals in biochemical reactions [1]. Our current finding that the xanthine oxidase/H_2O_2 system can generate $SO_3^{\bullet-}$ radical from SO_3^{2-} suggests a new property of this enzyme. Second, this result points to a new enzymatic pathway for sulfite metabolism and toxicity, since the $SO_3^{\bullet-}$ radical has been implicated in the mechanism of toxic reactions resulting from sulfite exposure [2–9]. Third, we noted that while the mechanism of $SO_3^{\bullet-}$ generation from SO_3^{2-} via autoxidation and trace-metal catalyzed oxidation has been studied in detail [10–14], relatively little is known about the mechanism of $SO_3^{\bullet-}$ generation through enzymatic pathway, with the exception of some recent studies [15–19]. In particular, Mason and coworkers [17–19] utilized ESR and ESR spin trap methodology to identify $SO_3^{\bullet-}$ formation during the prostaglandin/hydroperoxidase-catalyzed oxidation of SO_3^{2-} [17,18], and $SO_3^{\bullet-}/SO_4^{\bullet-}$ radical formation during SO_3^{2-} oxidation by peroxidase/H_2O_2 [19].

Correspondence address: N.S. Dalal, Department of Chemistry, West Virginia University, Morgantown, WV 26506, USA.

2. MATERIALS AND METHODS

The spin trap 5,5-dimethyl-1-pyrroline-1-oxide (DMPO) was purchased from Aldrich, and was purified by charcoal decolorization [20]. Xanthine oxidase (from bovine milk), DETAPAC (diethylenetriaminepenta acetic acid), and xanthine were purchased from Sigma. Catalase (from bovine liver) was purchased from Boehringer Mannheim. Hydrogen peroxide (H_2O_2), phosphate buffer (pH 7.2), and sodium sulfite (Na_2SO_3) were purchased from Fisher. All Na_2SO_3 solutions were freshly made in a phosphate buffer. Inactivation of xanthine oxidase was achieved by heating the enzyme in an oven at 90°C for 20 h.

All ESR spectra were obtained at the X-band (~9.5 GHz) frequencies using a Varian E3 or a Bruker ER 200D spectrometer, essentially as described earlier [21].

3. RESULTS

3.1. *$SO_3^{\bullet-}$ generation from SO_3^{2-} by xanthine oxidase/H_2O_2*

As mentioned in the Introduction, sulfite solutions generate $SO_3^{\bullet-}$ radical due to trace-metal ion catalyzed oxidation [10–14]. To prevent this, 2.0 mM DETAPAC was added to all of the reaction mixtures, following Mottley and Mason [19]. As may be noted from Fig. 1a, a barely detectable ESR signal was obtained when 10 mM Na_2SO_3, 60 mM DMPO and 2 mM DETAPAC were mixed in a phosphate buffer (pH 7.2). Addition of 0.1 mM H_2O_2 to this mixture did not significantly alter the signal intensity (Fig. 1b). However, a strong ESR signal was observed when xanthine oxidase, H_2O_2 and SO_3^{2-} were mixed in the presence of DMPO (Fig. 1c). The observed ESR spectrum was analyzed in terms of nitrogen (a_N) and hydrogen (a_H) hyperfine couplings. The analysis yielded a_N = 14.7 G and a_H = 16.0 G. These a_N and a_H values as well as the ratio of a_N-to-a_H (0.92) are nearly identical to those reported earlier for the DMPO/

Published by Elsevier Science Publishers B.V.

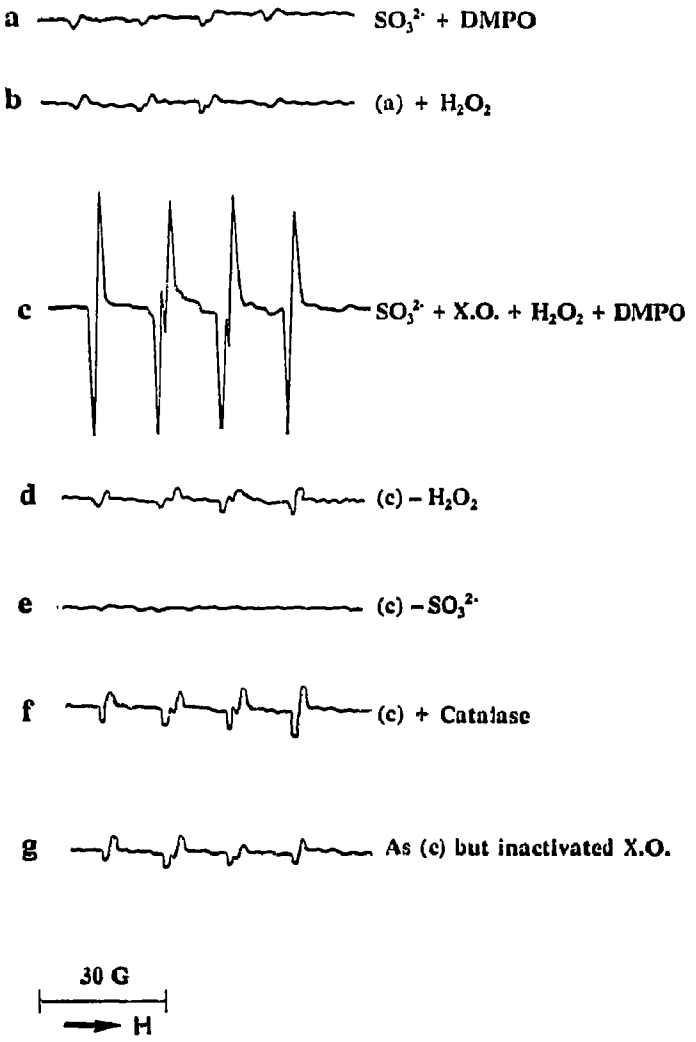

Fig. 1. ESR spectra recorded 1 min after mixing: (a) 10 mM Na_2SO_3, 60 mM DMPO and 2 mM DETAPAC in a pH 7.2 phosphate buffer; (b) same as (a) but with 0.1 mM H_2O_2; (c) same as (b) but with 1 unit/ml xanthine oxidase (X.O.); (d) same as (c) but without H_2O_2; (e) same as (c) but without Na_2SO_3; (f) same as (c) but with catalase; (g) same as (a) but with inactivated xanthine oxidase (X.O.). The spectrometer settings were: receiver gain, 1.25×10^5; modulation amplitude, 1.0 G; scan time, 4 min; time constant, 0.3 s.

$SO_3^{\bullet-}$ adduct formed in other systems [11,13,17,19,22–25], demonstrating the formation of the $SO_3^{\bullet-}$ radical in the reaction mixture. Omission of any one component caused a dramatic decrease in $SO_3^{\bullet-}$ generation (Fig. 1b, d and e). Also, addition of catalase caused a sharp decrease in the $SO_3^{\bullet-}$ generation (Fig. 1f). Thus the activity of xanthine oxidase for $SO_3^{\bullet-}$ generation from SO_3^{2-} appears to be driven by H_2O_2. Additional evidence for the role of H_2O_2 will be presented in section 3.3.

3.2. *Evidence for $SO_3^{\bullet-}$ generation being enzymatic*

Utilization of inactivated xanthine oxidase generated a very weak DMPO/$SO_3^{\bullet-}$ signal (Fig. 1g), essentially

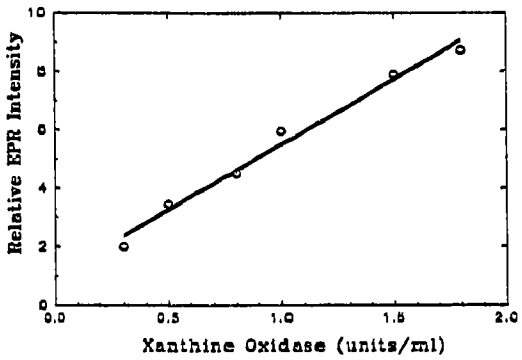

Fig. 2. Effect of xanthine oxidase (X.O.) concentration on $SO_3^{\bullet-}$ generation. The reaction mixture containing in a pH 7.2 phosphate buffer, 60 mM DMPO, 2 mM DETAPAC, 10 mM Na_2SO_3, 0.1 mM H_2O_2 and various X.O. concentrations.

similar to that obtained in the absence of xanthine oxidase (Fig. 1b), suggesting an enzymatic mechanism for the $SO_3^{\bullet-}$ generation process. It is known that an essential feature of an enzyme-catalyzed reaction is that the initial rate of product formation is linearly proportional to the enzyme concentration when the substrate concentration greatly exceeds the enzyme concentration [26]. Indeed, the amount of $SO_3^{\bullet-}$ generated was found to in-

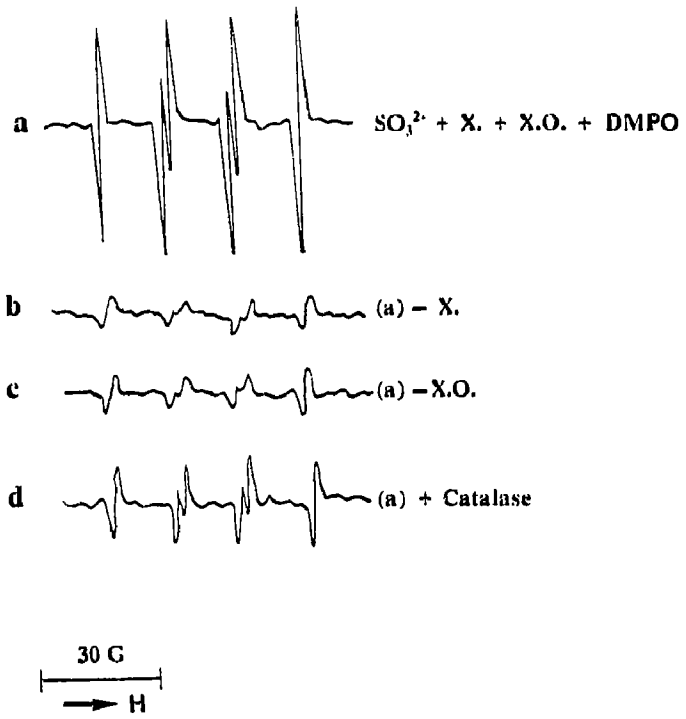

Fig. 3. ESR spectra recorded 1 min after mixing: (a) 10 mM Na_2SO_3, 60 mM DMPO, 0.1 mM H_2O_2, 1 unit/ml xanthine oxidase (X.O.) and 2 mM DETAPAC in a pH 7.2 phosphate buffer; (b) same as (a) but without xanthine (X.); (c) same as (a) but without xanthine oxidase (X.O.); (d) same as (a) but with catalase. The spectrometer settings were the same as those in the legend to Fig. 1.

crease fairly linearly with the xanthine oxidase concentration (Fig. 2), supporting the conjecture that the $SO_3^{\bullet-}$ radical generation results from an enzyme mediated reaction.

3.3. *Additional evidence for H_2O_2 participation*

The xanthine oxidase/xanthine system was utilized to evaluate the role of H_2O_2 in $SO_3^{\bullet-}$ generation from SO_3^{2-} by xanthine oxidase. It is known that the xanthine oxidase/xanthine system produces both H_2O_2 and O_2^- [27–30]. As shown in Fig. 3a, a reaction mixture of xanthine, xanthine oxidase, SO_3^{2-}, and DMPO generates a strong spin adduct signal. Its similarity in the spectral lineshape and hyperfine splittings to those in Fig. 1c implies that the spin adducts are DMPO/$SO_3^{\bullet-}$. Omission of either xanthine or xanthine oxidase resulted in a substantial reduction in the spectral intensity (Fig. 3b and c), indicating the necessity of both xanthine and xanthine oxidase for the $SO_3^{\bullet-}$ formation. Addition of catalase caused significant decrease in the signal intensity (Fig. 3d). It can thus be deduced that xanthine oxidase and H_2O_2 (produced by xanthine oxidase/xanthine system) generate the $SO_3^{\bullet-}$ radical.

4. DISCUSSION

The above spin trapping measurements demonstrate that, in the presence of H_2O_2, xanthine oxidase can catalyze $SO_3^{\bullet-}$ formation from SO_3^{2-} enzymatically. This result has at least two significant implications: (i) it suggests a new property for xanthine oxidase; and (ii) it provides a possible new metabolic pathway for $SO_3^{\bullet-}$ generation in cellular systems, where both H_2O_2 and xanthine oxidase are present. As mentioned in the Introduction, it is generally believed that $SO_3^{\bullet-}$ radicals play an important role in the biochemical mechanism of SO_3^{2-} toxicity [2–9]. For example, $SO_3^{\bullet-}$ radicals are known to cause many adverse reactions with biological molecules, including methionine and diphosphopyridine nucleotide oxidation [8,31], β-carotene and tryptophan destruction [7,8], double bond addition in alkenes [32], fatty acid peroxidation [33], nucleic acid modification [3], and DNA cleavage [34]. Since the $SO_3^{\bullet-}$ generation from SO_3^{2-} autoxidation is not thought to occur significantly in vivo, the generation of $SO_3^{\bullet-}$ by enzyme-mediated reactions may be important [15,16]. It has also been suggested [35] that $SO_3^{\bullet-}$ generation via enzymatic pathway may be responsible for some of the injuries which result to humans deficient in sulfite oxidase. Thus $SO_3^{\bullet-}$ generation by xanthine oxidase/H_2O_2 may play an important role in the mechanism of SO_3^{2-} related toxicity.

While additional investigations are needed to understand the mechanism of $SO_3^{\bullet-}$ generation by xanthine oxidase/H_2O_2, the dependence of the reaction on H_2O_2 suggests that H_2O_2 binds to xanthine oxidase to generate a xanthine oxidase–H_2O_2 complex, which in turn reacts with SO_3^{2-} to produce $SO_3^{\bullet-}$ radicals. This mechanism for the generation of $SO_3^{\bullet-}$ radicals by xanthine oxidase/H_2O_2 is essentially analogous to that proposed earlier for the horseradish peroxidase/H_2O_2 system [35].

In summary, this communication reports on the observation of a new property of xanthine oxidase – that in the presence of H_2O_2, it can catalyze the generation of $SO_3^{\bullet-}$ radical from SO_3^{2-}. Even though we have not yet elucidated the underlying biochemical mechanism, this result itself should be of significance in studies dealing with the metabolism and toxicity of sulfite, because xanthine oxidase is sometimes used as an enzyme for generating O_2^- in studies of sulfite reactivity [36]. Further studies aimed at understanding the chemical nature of the active site for this reaction should improve our understanding of the mechanism of $SO_3^{\bullet-}$ generation by this enzyme.

Acknowledgement: This research was supported in part by the National Institutes of Occupational Safety and Health Grant U60-CCU306149-01-1.

REFERENCES

[1] Hughes, M.N. (1982) The Inorganic Chemistry of Biological Processes, p. 81 and pp. 172–176, Wiley, Chichester, UK.
[2] Brestel, E.P., Perrone, W.F. and Cohen, R.A. (1986) J. Free Radical Biol. Med. 2, 218–225.
[3] Hayatsu, H. (1976) Prog. Nucleic Acid Res. Mol. Biol. 16, 75–124.
[4] Inouye, B., Ikeda, M., Ishida, T., Ogata, M., Akiyama, J. and Utsumi, K. (1978) Toxicol. Appl. Pharmacol. 46, 29–38.
[5] Kaplan, D., McJilton, C. and Luchtel, D. (1975) Arch. Environ. Health 30, 507–509.
[6] Norman, R.O.C. and Storey, P.M. (1971) J. Chem. Soc. (B) 1009–1013.
[7] Peiser, G.D. and Yang, S.F. (1979) J. Agric. Fd. Chem. 27, 446–449.
[8] Yang, S.F. (1970) Biochemistry 9, 5008–5014.
[9] Yang, S.F. (1973) Environ. Res. 6, 395–402.
[10] Neta, P. and Huie, R.E. (1985) Environ. Health Persp. 64, 209–217.
[11] Reed, G.A., Curtis, J.F., Mottley, C., Eling, T.E. and Mason, R.P. (1986) Proc. Natl. Acad. Sci. USA 83, 7499–7502.
[12] Ito, K. and Kawanishi, S. (1991) Biochem. Biophys. Res. Commun. 178, 1306–1321.
[13] Shi, X. and Dalal, N.S. (1990) Res. Chem. Intermediates 13, 103–115.
[14] Ozawa, T. and Kwan, T. (1985) Tetrahedron 4, 1995–1996.
[15] Gunnison, A.F. (1981) Fd. Cosmet. Toxicol. 19, 667–662.
[16] Mottley, C. and Mason, R.P. (1989) Biol. Mag. Reson. 8, 489–546.
[17] Mottley, C., Mason, R.P., Chignell, C.F., Sivarajah, K. and Eling, T.E. (1982) J. Biol. Chem. 257, 5050–5055.
[18] Mottley, C., Trice, T.B. and Mason, R.P. (1982) Mol. Pharmacol. 22, 732–737.
[19] Mottley, C. and Mason, R.P. (1988) Arch. Biochem. Biophys. 267, 681–689.
[20] Buettner, G.R. and Oberley, L.W. (1974) Biochem. Biophys. Res. Commun. 83, 69–74.
[21] Shi, X., Sun, X. and Dalal, N.S. (1990) FEBS Lett. 271, 185–188.
[22] Covello, P.C. and Thompson, J.E. (1985) Biochim. Biophys. Acta 843, 150–154.

[23] Lambeth, D.O. and Palmer, G. (1973) J. Biol. Chem. 248, 6095–6013.
[24] Dalal, N.S. and Shi, X. (1989) Biochemistry 28, 748–750.
[25] Li, A.S.W. and Chignell, C.F. (1991) J. Biochem. Biophys. Methods 22, 83–87.
[26] Ottaway, J.H. and Apps, D.K. (1984) Biochemistry, pp. 24–44, Baillière Tindall Press, London.
[27] Freeman, B. and Crapo, J. (1982) Lab. Invest. 47, 412–426.
[28] Weiss, S.J. and LoBuglio, A.F. (1982) Lab. Invest. 47, 5–18.
[29] Haber, F. and Weiss, J. (1934) Proc. R. Soc. London A 147, 332–351.
[30] McCord, J.M. and Fridovich, I. (1968) J. Bio. Chem. 243, 5753–5760.
[31] Klebanoff, S.J. (1961) Biochim. Biophys. Acta 48, 93–103.
[32] Southerland, W.M., Akogyeram, C.O., Toghrol, F., Sloan, L. and Scherrer, R. (1982) J. Toxicol. Environ. Health 10, 479–491.
[33] Lizada, M.C.C. and Yang, S.F. (1981) Lipids 16, 189–194.
[34] Hayatsu, H. and Miller, R.C. (1972) Biochem. Biophys. Res. Commun. 46, 120–124.
[35] Mason, R.P., Chignell, C.F., Eling, T.E. and Mottley, C. (1984) Life Chemistry Reports Suppl. 2, pp. 55–63, Harwood Academic Publishers, Chur, Switzerland.
[36] McCord, J.M. and Fridovich, I. (1969) J. Biol. Chem. 244, 6056–6063.