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8-Hydroxy-2'-deoxyguanosine formation and DNA damage induced by sulfur trioxide anion radicals

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Abstract: The 8-hydroxy-2'-deoxyguanosine (8-OHdG) formation and DNA damage by sulfur trioxide anion radicals ($SO_3^{\cdot-}$) were investigated using ESR spin trapping, HPLC, and electrophoretic assays. Sulfite (SO_3^{2-}) autoxidation generated both hydroxyl ($^{\cdot}OH$) and $SO_3^{\cdot-}$ radicals. Oxidation of SO_3^{2-} by chromium(VI) generated only $SO_3^{\cdot-}$ with much enhanced yield. Incubation of 2'-deoxyguanosine (dG) with SO_3^{2-} generated 8-OHdG albeit at low yield. Chromium(VI) enhanced the yield four-fold. Electrophoretic assays showed that $SO_3^{\cdot-}$ radicals generated by chromium(VI) oxidation of SO_3^{2-} caused DNA double strand breaks. The results demonstrate that $SO_3^{\cdot-}$ radicals are capable of causing dG hydroxylation and DNA double strand breaks.

Sulfite (SO_3^{2-}) exposure can cause toxic effects, such as the induction of an acute asthmatic state (1, 2). SO_3^{2-} also appears to have adverse genetic effects (2) and can act as a mutagen or comutagen and a cocarcinogen (1-3). While the exact mechanism of SO_3^{2-} toxicity is not yet fully understood, it is generally thought to be related to SO_3^{2-} oxidation process, involving, in particular, $SO_3^{\cdot-}$ radicals (4-6). For example, $SO_3^{\cdot-}$ radicals are reported to cause methionine and diphosphopyridine nucleotide oxidation (7-8), β-carotene and tryptophan destruction (7, 9), double bond addition in alkene (10). Since $SO_3^{\cdot-}$ can be generated in cellular system by autoxidation (3, 11) and by enzyme-mediated oxidation of SO_3^{2-} (12, 13), the possible interaction of $SO_3^{\cdot-}$

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toward DNA appears to be important in understanding the mechanism of SO_3^{2-}-related cellular damage.

The present investigation was undertaken to examine the following questions: (a) Does $SO_3^{\cdot-}$ cause 2'-deoxyguanosine (dG) hydroxylation to generate 8-hydroxy-2'-deoxyguanosine (8-OHdG)? (b) Does $SO_3^{\cdot-}$ cause DNA double strand breaks.

Materials and Methods

dG, $K_2Cr_2O_7$, Na_2SO_3, ethanol, 5,5-dimethyl-1-pyrroline N-oxide (DMPO), and sodium formate were purchased from Sigma Chemical Company (St. Louis, MO). DNA (λHind III digest marker fragments) were obtained from Bethesda Research Laboratories (Gaithersburg, MD). The reference 8-OHdG was synthesized by Dr. Peter M. Gannett, Department of Basic Pharmaceutic Sciences, West Virginia University (Morgantown, WV). Chelex-100 was obtained from Bio-Rad Laboratories (Richmond, CA). All solutions were prepared in phosphate buffer (pH 7.4) which had been purified by treatment with Chelex-100.

HPLC measurements were made using an Alterx ODS C^{18} column (4.6 x 250 nm) equilibrated with 50 mM KH_2PO_4/7% methanol at a flow rate of 1.5 ml/min. The 8-OHdG formation was monitored at 254 nm by using electrochemical detector (ECD) (model LC 4422030, Bio-Analytical Systems) (West Lafayette, IN) set at 600 mV and 5-50 nA full scale. Under these conditions the retention time was 8.6 minutes for dG and 12.3 minutes for 8-OHdG. The identity of the 8-OHdG peak in experimental sample was confirmed using a sample containing an added 8-OHdG reference standard. The HPLC system was calibrated with a standard solution of dG and 8-OHdG. All results are presented as means of at least duplicate experiments, which showed only minor variation from each other.

ESR spin trapping (14, 15) was employed for detecting short-lived free radical intermediates. All ESR measurements were made utilizing a Varian E4 ESR spectrometer and a flat cell assembly. Hyperfine couplings were measured (to 0.1 G) directly from magnetic field separations using 1,1-diphenyl-2-picryhydrazyl (DPPH) and potassium tetraperoxochromate (K_3CrO_8) as reference standards.

The DNA double strand breakage assay was carried out according to the method described earlier (16). Briefly, reactions were carried out in 10 mM phosphate buffer (pH 7.4) in 1.5 ml polypropylene tube at 37°C. Each reaction mixture contained 10 μg DNA in a total volume of 100 μl. DNA damage was assayed for each reaction by removal of a 10 μl aliquot. To this solution 2 μl of gel loading buffer (50 mM EDTA, 2.5% sodium dodecyl sulfate, 0.1% bromophenol blue, and 6.25% glycerol) was added and then the sample was electrophoresed in 0.7% agarose at 1-2 V/cm in 40 mM Tris-acetate buffer containing 2 mM EDTA (pH 8.0) for 6 hours and photographed under ultraviolet transillumination.

All experiments were performed at room temperature in ambient air except those specifically indicated.

Results

Free radical generation

Figure 1a shows an ESR spectrum obtained from a mixture of SO_3^{2-} and DMPO at physiological pH (pH 7.4). The analysis of this spectrum yields hyperfine splittings of a_N = 14.7 G and a_H = 16.0 G, which were identical to those reported earlier for the DMPO/$SO_3^{\cdot-}$ adduct formed in various other reaction systems (3, 11-13, 17). The spectrum in Figure 1a shows the formation of $SO_3^{\cdot-}$ radicals in the autoxidation of SO_3^{2-}, in agreement with earlier report (3).

It has been suggested that SO_3^{2-} autoxidation generated not only $SO_3^{\cdot-}$ radicals but also hydroxyl radicals ($^{\cdot}OH$) (11, 18). Since the hyperfine splittings of DMPO/$^{\cdot}OH$ are $a_N = a_H$ = 15.0 G, the spin adduct signals of DMPO/$^{\cdot}OH$ may overlap with DMPO/$SO_3^{\cdot-}$. To detect the possible $^{\cdot}OH$ generation, $^{\cdot}OH$ radical scavenger, ethanol was added to the mixture of SO_3^{2-} and DMPO. It is known

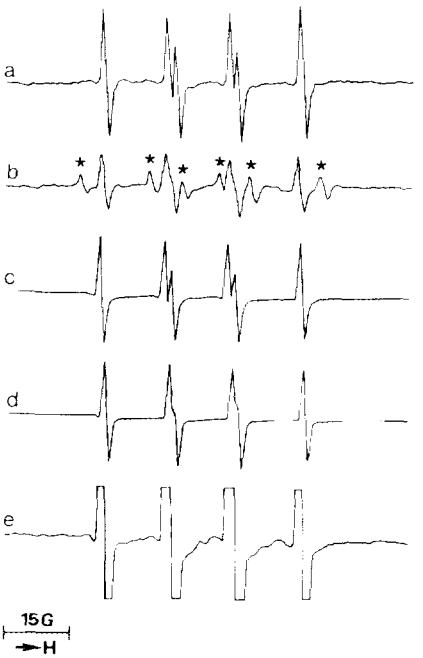

Figure 1. (a) ESR spectrum recorded 5 minutes after mixing of 10 mM Na_2SO_3 and 100 mM DMPO in a phosphate buffer solution (pH 7.4). (b) Same as (a) but with 30% ethanol added. (c) Same as (a) but with 2 mM $K_2Cr_2O_7$ added. (d) Same as (a) but with 2 mM $K_2Cr_2O_7$ and 30% ethanol added. (e) Same as (d) but using higher receiver gain. The spectrometer settings were modulation amplitude, 1 G; field, 3470 ± 100 G; receiver gain, 1.25 x 10^5 for (a), (b) and (e), 2.0 x 10^4 for (c) and (d). The asterisks indicate peaks for DMPO/$^{\cdot}CHOHCH_3$ adduct.

that the ·OH radical abstracts a hydrogen atom from ethanol with the formation of a new radical (19). This newly generated radical will be trapped by DMPO to generate a new spin adduct signal. As expected, addition of ethanol (30%) results in the appearance of a new spin adduct signal (as indicated by asterisks in Figure 1b) with the hyperfine splittings of a_N = 15.8 G and a_H = 22.8 G. These splittings are typical of those of DMPO/·$CHOHCH_3$ adduct (17). The results demonstrate that SO_3^{2-} autoxidation indeed generates ·OH radicals.

The oxidation of SO_3^{2-} by chromium(VI) was employed as a source of pure $SO_3^{\cdot-}$ radicals. As shown in Figure 1c, addition of Cr(VI) to an incubation mixture of SO_3^{2-} and DMPO generated a strong DMPO/$SO_3^{\cdot-}$ signal (Figure 1d). Addition of ethanol (30%) did not effect the signal intensity (Figure 1d). A small decrease in a_H splitting can be contributed to a solvent effect. The spectrum of Figure 1e was recorded under the same conditions as in Figure 1d except under a higher receiver gain. Since the spectra in Figures 1d and 1e did not exhibit any additional peaks upon addition of ethanol, it can be concluded that oxidation of SO_3^{2-} by chromium(VI) did not generate any significant amount of ·OH radicals. The results also indicate that $SO_3^{\cdot-}$ radicals do not significantly react with ethanol to generate ethanol-derived free radicals.

8-OHdG formation

Figure 2 shows a representative chromatogram produced from a solution of 3 mM SO_3^{2-} and 1 mM dG in phosphate buffer (pH 7.4).

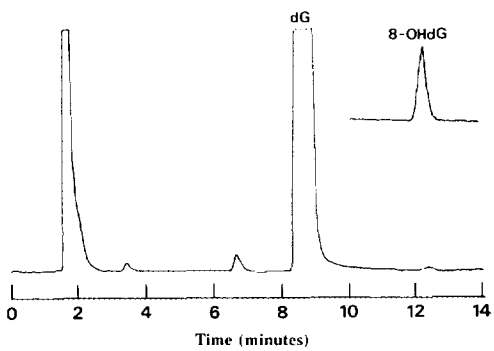

Figure 2. HPLC fraction of dG from incubation of 3 mM Na_2SO_3 and 1 mM dG. dG and 8-OHdG were eluted at 8.6 and 12.3 minutes, respectively. Lower part shows UV (A_{254}) and upper part shows electrochemical detection. The sample was injected after 40 minutes of incubation at room temperature under ambient air.

Table 1
8-OHdG formation from dG hydroxylation by SO_3^{2-} reaction

Reaction mixture[a]	8-OHdG/dG [ng/μg ± SE (n = 3)]
SO_3^{2-} + dG	1.11 ± 0.051
SO_3^{2-} + dG + $K_2Cr_2O_7$	4.61 ± 0.112
SO_3^{2-} + dG + $K_2Cr_2O_7$ + EtOH	0.24 ± 0.021
SO_3^{2-} + dG + $K_2Cr_2O_7$ + formate	0.36 ± 0.032

[a]Concentrations of reactants in the reaction mixtures were as follows: dG, 1.0 mM; SO_3^{2-}, 3 mM; $K_2Cr_2O_4$, 0.4 mM; EtOH, 50 mM; formate, 50 mM. The reaction mixtures were incubated in phosphate buffer (pH 7.4) at room temperature for 40 minutes under ambient air.

8-OHdG eluted at 12.3 minutes as a well resolved peak. Inclusion of synthetic 8-OHdG in the sample increased the peak intensity but did not alter the elution time. The upper part of Figure 2 represents electrochemical detection and the lower part UV (A_{254}) detection. Both of these generate 8-OHdG peaks with the same elution time, confirming 8-OHdG formation in the incubation mixture.

As shown in Table 1, chromium(VI) enhanced 8-OHdG formation about four-fold. Both ethanol and formate inhibited 8-OHdG formation.

DNA double strand breaks

Figure 3, lane 1 shows DNA alone as a control. Incubation of DNA with SO_3^{2-} for 4 hours induced little , if any, DNA damage (Figure 3, lane 2). Incubation of DNA with SO_3^{2-} and chromium(VI) caused DNA double strand breaks (Figure 3, lane 3). Both ethanol and formate inhibited the DNA damage (Figure 3, lanes 4 and 5).

Discussion

The results show that $SO_3^{\cdot-}$ radicals are capable of causing dG hydroxylation to generate 8-OHdG and causing DNA double strand break. The following observations support this conclusion. (a) SO_3^{2-} autoxidation generated both $SO_3^{\cdot-}$ and $^{\cdot}OH$ radicals. Incubation of SO_3^{2-} with dG caused a relatively low yield of 8-OHdG. Oxidation of SO_3^{2-} by chromium(VI) generated a much higher yield of $SO_3^{\cdot-}$ but did not generate any detectable amount of $^{\cdot}OH$

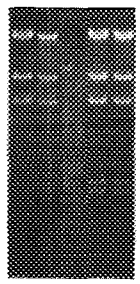

Figure 3. DNA double strand breaks by SO_3^{2-} reactions. Lane 1, DNA alone; lane 2, DNA + 0.5 mM Na_2SO_3; lane 3, DNA + 0.5 mM Na_2SO_3 + 0.5 mM $K_2Cr_2O_7$; lane 4, same as lane 3, but with 50 mM ethanol added; lane 5, same as lane 3, but with 50 mM sodium formate added. The samples were incubated for 24 hours. Other experimental conditions were discribed in the section of Materials and Methods.

radicals. Incubation of SO_3^{2-} and chromium(VI) with dG generated a much higher yield of 8-OHdG. Thus the yield of 8-OHdG formation is correlated to the amount of $SO_3^{\cdot-}$ radicals and not $^{\cdot}OH$ radicals. (b) Incubation of SO_3^{2-} with DNA for 4 hours caused little, if any, DNA damage while incubation of SO_3^{2-} and chromium with DNA caused a much higher level of DNA double strand breaks. Since oxidation of SO_3^{2-} by chromium(VI) did not generated any detectable amount of $^{\cdot}OH$ radicals but a high yield of $SO_3^{\cdot-}$ radicals, it is the $SO_3^{\cdot-}$ radicals and not the $^{\cdot}OH$ radicals that play a key role in causing the DNA double strand breaks.

It has been reported that $^{\cdot}OH$ radicals cause dG hydroxylation to generate 8-OHdG (20). Recently, it has been reported that singlet oxygen, $^1O_2$, is also able to generate 8-OHdG from dG. The results in this study show that $SO_3^{\cdot-}$ radicals also have this property. While additional studies are necessary to establish its mechanism, the following reaction steps may best account for the results obtained.

$$dG + SO_3^{\cdot-} \text{ ---> } dG^{+\cdot} + SO_3^{2-} \qquad [a]$$

$$dG^{+\cdot} + H_2O \text{ ---> } dGOH^{\cdot} + SO_3^{2-} + H^+ \qquad [b]$$

$$dGOH^{\cdot} + SO_3^{\cdot-} \text{ ---> } dGO \text{ (8-OHdG)} + SO_3^{2-} + H^+ \qquad [c]$$

Due to their reactive nature, the reactive intermediates, $dG^{+\cdot}$ and $dGOH^{\cdot}$, will react with ethanol or formate. The inhibitory

effect of ethanol and formate on both 8-OHdG formation and DNA double strand breaks may be due to the scavenging effect of these intermediates by ethanol and formate.

In summary, the present study demonstrates that (a) SO_3^{2-} autoxidation generates both $SO_3^{\cdot-}$ and $^{\cdot}OH$ radicals. (b) SO_3^{2-} oxidation by chromium(VI) generates a higher level of $SO_3^{\cdot-}$ radicals. This reaction does not generate any detectable amount of $^{\cdot}OH$ radicals. (c) $SO_3^{\cdot-}$ radicals are capable of causing dG hydroxylation to generate 8-OHdG. (d) $SO_3^{\cdot-}$ radicals cause DNA double strand breaks. These results may improve our understanding on the SO_3^{2-} toxicity and carcinogenicity.

References

1. Gunnison, A.F. (1981) Fd. Cosmet. Toxicol. 19, 667-682.
2. Shapiro, R. (1977) Mutat. Res. 39, 149-176.
3. Reed, G.A., Curtis, J.F., Mottley, C., Eling, T.E., and Mason, R.P. (1986) Proc. Natl. Acad. Sci. USA 83, 7499-7502.
4. Brestel, E.R., Petrone, W.F., and Cohen, R.A. (1986) J. Free Radical Biol. Med. 2, 219-225.
5. Inouye, B., Ikeda, M., Ishida, T., Ogata, M., Akiyama, J., and Utsumi, K. (1978) Toxicol. Appl. Pharmacol. 46, 29-38.
6. Yang, S.F. (1973) Environ. Res. 6, 395-402.
7. Yang, S.F. (1970) Biochemistry 9, 5008-5014.
8. Klebanoff, S.J. (1961) Biochim. Biophys. Acta 48, 93-103.
9. Peiser, G.D., and Yang, S.F. (1979) J. Agric Fd. Chem. 27, 446-449.
10. Southerland, W.M., Akogyeram, C.O., Toghrol, F., Sloan, L., and Scherrer, R. (1982) J. Toxicol. Environ. Health 10, 479-491.
11. Shi, X., and Dalal, N.S. (1990) Res. Chem. Intermediates 13, 103-105.
12. Mottley, C., Mason, R.P., Chignell, C.F., Sivarajah, K., and Eling, T.E. (1982) J. Biol. Chem. 257, 5050-5055.
13. Sun, X., Shi, X., and Dalal, N.S. (1992) FEBS Lett. 303, 213-216.
14. Janzen, E.G., and Blackburn, B.J. (1968) J. Am. Chem. Soc. 90, 5909-5910.
15. Mottley, C., and Mason, R.P. (1989) Biol. Magn. Reson. 8, 489-546.
16. Daniel, L.N., Mao, Y., and Saffiotti, U. (1992) Free Radical Biol. Med. 14, 463-472.
17. Buettner, G.R. (1987) Free Radical Biol. Med. 3, 259-303.
18. Thompson, J.E., and Covello, P.S. (1985) Biochim. Biophys. Acta 843, 150-154.
19. Shi, X., Dong, Z., Dalal, N.S., and Gannett, P.M. (1994) Biochim. Biophys. Acta 1226, 65-72.
20. Floyd, R.A., West, J.J., Wong, P.K., Altimiller, D.H., and Tingey, D.T. (1986) Free Radical Res. Commun. 1, 163-172.
21. Devasagayam, T.P.A., Steenkem, S., Obendorf, M.S.W., Schult, W.A., and Sies, H. (1991) Biochemistry 30, 6283-6289.