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Hydroxylation of Deoxyguanosine at the C-8 Position in the Thiosulfate-Hydrogen Peroxide Reaction System. Evidence of Hydroxyl Radical Generation in the System

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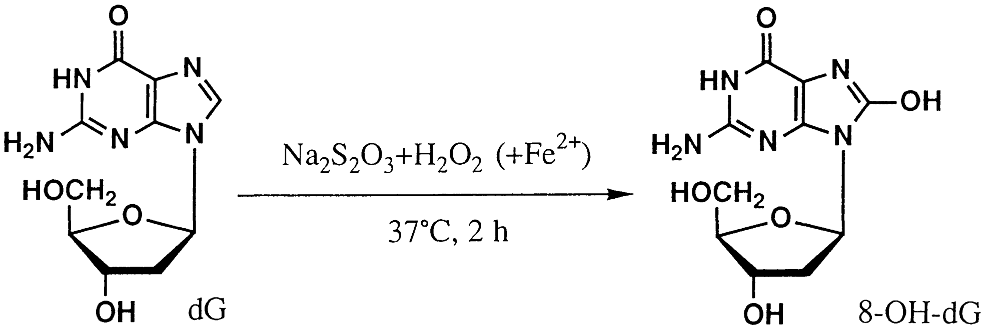
The thiosulfate-hydrogen peroxide reaction system generates thiosulfate radical anion and hydroxyl radical. When 2'-deoxyguanosine (dG) was included in the system, it was hydroxylated at the C-8 position, and the hydroxylation was markedly enhanced by addition of a trace amount of iron ion.

It is known that 8-hydroxydeoxyguanosine (8-OH-dG)[1] is a marker of DNA damage caused by active oxygen species both *in vitro*[2] and *in vivo*.[3] Hydroxylation of guanine base at the C-8 position occurs in hydroxyl radical-generating systems such as the Udenfriend and the Fenton systems.[4] Cohen and Spencer[5] have found that, of the possible reactions between sodium thiosulfate ($Na_2S_2O_3$) and hydrogen peroxide (H_2O_2), the one which predominates is

$$2\ Na_2S_2O_3 + 4\ H_2O_2 \rightarrow Na_2S_3O_6 + Na_2SO_4 + 4\ H_2O \qquad (1)$$

Recently, Ozawa and Hanaki[6] reported formation of thiosulfate radical anion ($S_2O_3^{\cdot-}$) in the system. But they did not mention hydroxyl radical formation. We found that when 2'-deoxyguanosine (dG) was included in the above system, 8-OH-dG was formed. We also observed a prolonged formation of hydroxyl radical (HO·) in the system.



An aqueous solution of 2'-deoxyguanosine (4 mM, 2 ml) containing 100 mM H_2O_2 and 34 mM $Na_2S_2O_3$ was incubated at 37℃ for 2 h in the dark (1 M = 1 mol dm^{-3}). In some experiments, iron (II) sulfate ($FeSO_4$) was added at 2, 4 or 6 μ M. All concentrations were expressed as final concentration in the reaction vessel. The amount of 8-OH-dG formed in the solution was measured by using a high-performance liquid chromatograph equipped with an electrochemical detector, as reported previously.7) A mixture of 100 mM H_2O_2 and 34 mM $Na_2S_2O_3$ was prepared for ESR examination. DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide) solution (50 μ l, 1 M conc.) was added to an aliquot (200 μ l) of the mixture. The time of DMPO addition was varied from 1 min to 3 h after mixing of $Na_2S_2O_3$ and H_2O_2. The mixture was transferred to a flat cell and an ESR spectrum was recorded at room temperature using a JEOL JES-RE1X spectrometer.

The ESR spectra of radical adducts of the spin trap DMPO are shown in Fig. 1. The radical ($S_2O_3^{\cdot -}$, Fig. 1-A) was formed in the early stage of the $Na_2S_2O_3$-H_2O_2 reaction. The hyperfine couplings were closely resemble to those of $SO_3^{\cdot -}$ 8) (a^H=1.60 mT and a^N=1.45 mT). The radical formed in the next stage was identified as HO· based on the good agreement of the parameters with those reported9) (Fig. 1-B, a^H=1.48 mT and a^N=1.48 mT). These results indicate that the production of $S_2O_3^{\cdot -}$ diminishes as the reaction proceeds. Instead of $S_2O_3^{\cdot -}$, HO· became the predominant radical in the solution. The production of HO· probably started early, because the absorption of *p*-nitrosodimethylaniline at 438 nm was decreased by addition of the freshly prepared $Na_2S_2O_3$-H_2O_2 solution. On the other hand, DMPO spin adduct of $SO_3^{\cdot -}$ formed during photolysis of Na_2SO_3 was detectable by ESR, whereas HO· was not detectable.8)

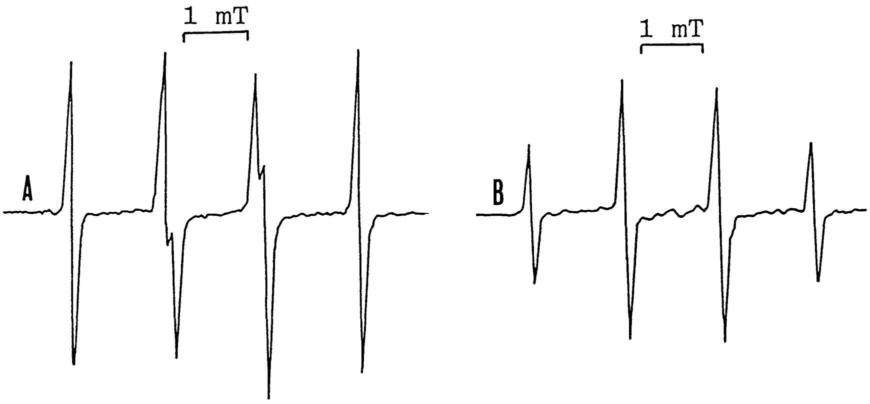


Fig. 1. The ESR spectra of DMPO-trapped radicals produced in the $Na_2S_2O_3$-H_2O_2 system. The spectrum was recorded 2 min after the start of incubation of 34 mM $Na_2S_2O_3$, 100 mM H_2O_2, and 200 mM DMPO at room temperature. DMPO was added 1 min (line A) or 3 h (line B) after mixing of $Na_2S_2O_3$ and H_2O_2.

Spectral data (UV λmax 245, 293 nm, EI-MS TMS deriv. M^+ *m/z* 643) of the product were in agreement with those of authentic 8-OH-dG.$^{4)}$ Table 1 shows that 8-OH-dG was formed from dG in the $Na_2S_2O_3$-H_2O_2 system. When iron (II) ion was added to the solution, a remarkable increase of 8-OH-dG formation was observed. However, the Fe^{2+}-H_2O_2 system (Table 1, E,F,G) gave only trace amounts of 8-OH-dG. The molar concentration ratio of $Na_2S_2O_3$:H_2O_2 was of critical importance for the yield of 8-OH-dG; the optimum ratio was found to be from 1:3 to 1:4 when the concentration of H_2O_2 was kept constant. This molar ratio is different from that of equation (1). Addition of catalase, superoxide dismutase, desferroxamine mesylate, D-mannitol or ethyl alcohol suppressed the formation of 8-OH-dG in a dose-dependent manner. Superoxide radical anion was not detected by means of the blue color development test with nitro blue tetrazolium.$^{10)}$ These results suggest that the hydroxylation of dG was caused by hydroxyl radical formed in the system. The conversion of dG to 8-OH-dG$^{11)}$ depended on temperature, time and concentrations of dG, $Na_2S_2O_3$ and H_2O_2. Iron ion, a common contaminant in commercial chemicals, accelerated 8-OH-dG formation in the system.

Table 1. Conversion of dG to 8-OH-dG in $Na_2S_2O_3$-H_2O_2 systems$^{a)}$

Entry	Reaction conditions	Yield / %
A	$Na_2S_2O_3$ + H_2O_2	0.6
B	$Na_2S_2O_3$ + H_2O_2 + 2μ M $FeSO_4$	2.1
C	$Na_2S_2O_3$ + H_2O_2 + 4μ M $FeSO_4$	2.5
D	$Na_2S_2O_3$ + H_2O_2 + 6μ M $FeSO_4$	2.6
E	H_2O_2 + 2μ M $FeSO_4$	0.002
F	H_2O_2 + 4μ M $FeSO_4$	0.004
G	H_2O_2 + 6μ M $FeSO_4$	0.007
H	$Na_2S_2O_3$	<0.001

a) Each reaction was performed in an aqueous solution containing 4 mM dG. Concentrations of $Na_2S_2O_3$ and H_2O_2 were 34 mM and 100 mM, respectively.

Thiosulfate radical anion may arise in the $Na_2S_2O_3$-H_2O_2 reaction as follows.$^{6)}$

$$S_2O_3^{2-} + H_2O_2 \rightarrow S_2O_3^{\cdot-} + HO\cdot + OH^- \qquad (2)$$

Sulfur trioxide radical anion may arise from sulfite in the similar manner.$^{6,12)}$ Although sodium sulfite solution (8 mM) converted dG to 8-OH-dG (yield 0.5% at 37 ℃ for 24 h, in the dark), the formation of 8-OH-dG was suppressed (yield 0.03%) when hydrogen peroxide was present in the solution. These results indicate that the Na_2SO_3-H_2O_2 system provides less HO· generation. So the $Na_2S_2O_3$-H_2O_2 system is a unique system generating hydroxyl radical. However, equation (2) does not explain the prolonged formation of hydroxyl radical. It is considered that $Na_2S_2O_3$ generates a chemical species which accelerates the Fenton reaction. When $Na_2S_2O_3$ was titrated with H_2O_2, pH of the solution drastically changed: neutral→ alkaline→ neutral→ acidic. The end point expressed as a molar ratio of $Na_2S_2O_3:H_2O_2$ was 1:4.

We propose a reaction mechanism to elucidate our results.

The first stage (from neutral to alkaline):

$$S_2O_3^{2-} + H_2O_2 \rightarrow S_2O_3^{\cdot -} + HO\cdot + OH^- \tag{2}$$

This reaction involves catalytic effect of iron ion:

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO\cdot + OH^- \tag{2a}$$

$$Fe^{3+} + S_2O_3^{2-} \rightarrow Fe^{2+} + S_2O_3^{\cdot -} \tag{2b}$$

$$S_2O_3^{2-} + HO\cdot \rightarrow S_2O_3^{\cdot -} + OH^- \tag{3}$$

The second stage (from alkaline to acidic):

$$S_2O_3^{\cdot -} + 2H_2O_2 \rightarrow HO\cdot + OH^- + 2SO_2 + H_2O \tag{4}$$

$$2\ HO\cdot \rightarrow H_2O_2 \tag{5}$$

$$SO_2 + H_2O_2 \rightarrow 2H^+ + SO_4^{2-} \tag{6}$$

The total equation is given by summation of the equations (2,3,4,5,6):

$$S_2O_3^{2-} + 4H_2O_2 \rightarrow 2H^+ + 2SO_4^{2-} + 3H_2O \tag{7}$$

Sodium thiosulfate is administered as an antidote to victims of arsenic or mercury poisoning. The present results imply that usage of $Na_2S_2O_3$ for chemotherapy may lead to generation of $S_2O_3^{\cdot -}$ and $HO\cdot$ radicals *in vivo*, because the co-factors, iron ion and H_2O_2, normally exist in human organs.

References

1) The favored tautomeric structure of 8-OH-guanine is the 8-keto form, 7,8-dihydro-8-oxoguanine.
2) H.Kasai and S.Nishimura, *Environ. Health Perspect.*, 67, 111 (1986).
3) C.G.Fraga,M.K.Shigenaga,J.W.Park,P.Degan, and B.N.Ames, *Proc. Natl. Acad. Sci. USA,* 87, 4533 (1990).
4) H.Kasai and S.Nishimura, *Nucleic Acids Research*, 12, 2137 (1984).
5) W.C.Cohen and J.L.Spencer, *Chem. Eng. Progr.*, 58, 40 (1961).
6) T.Ozawa and A.Hanaki, *Biochem. Int.*, 20, 649 (1990).
7) H.Kasai, P.F.Crain, Y.Kuchino, S.Nishimura, A.Ootsuyama, and H.Tanooka, *Carcinogenesis*, 7, 1849 (1986).
8) C.F.Chignell, B.Kalyanaraman, R.H.Sik, and R.P.Mason, *Photochem. Photobiol.*, 34, 147 (1981).
9) G.R.Buettner, *Free Radical Biol.Med.*, 3, 259 (1987).
10) 3,3'-[3,3'-Dimethyl-(1,1'-biphenyl)-4,4'-diyl]-bis[2-(*p*-nitrophenyl)-5-phenyl-2*H* tetrazolium chloride].
11) Maximum yield was 8.1%. In this case, only 44.9% of deoxyguanosine was recovered. The major products were guanine (29.3%) and 8-hydroxyguanine (2.4%).
12) B.D.Flockhart, K.J.Ivin, R.C.Pink, and B.D.Sharma, *J. Chem. Soc., Chem. Commun.*, 1971, 339 (1971).

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