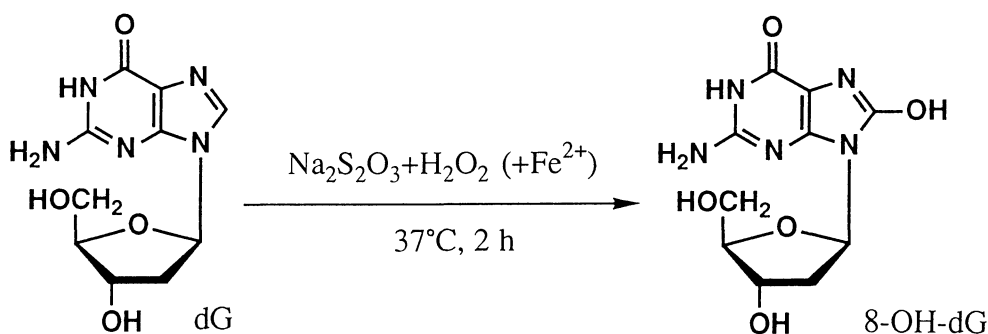
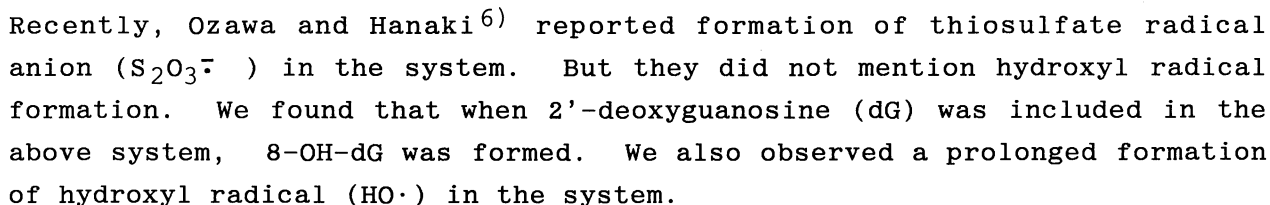


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It is known that 8-hydroxydeoxyguanosine (8-OH-dG)¹⁾ is a marker of DNA damage caused by active oxygen species both *in vitro*²⁾ and *in vivo*.³⁾ Hydroxylation of guanine base at the C-8 position occurs in hydroxyl radical-generating systems such as the Udenfriend and the Fenton systems.⁴⁾ Cohen and Spencer⁵⁾ have found that, of the possible reactions between sodium thiosulfate ($\text{Na}_2\text{S}_2\text{O}_3$) and hydrogen peroxide (H_2O_2), the one which predominates is



An aqueous solution of 2'-deoxyguanosine (4 mM, 2 ml) containing 100 mM H_2O_2 and 34 mM $\text{Na}_2\text{S}_2\text{O}_3$ was incubated at 37°C 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 μM . 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 $\text{Na}_2\text{S}_2\text{O}_3$ was prepared for ESR examination. DMPO (5,5-dimethyl-1-pyrroline-*N*-oxide) solution (50 μl , 1 M conc.) was added to an aliquot (200 μl) of the mixture. The time of DMPO addition was varied from 1 min to 3 h after mixing of $\text{Na}_2\text{S}_2\text{O}_3$ 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 ($\text{S}_2\text{O}_3^{\cdot -}$, Fig. 1-A) was formed in the early stage of the $\text{Na}_2\text{S}_2\text{O}_3$ - H_2O_2 reaction. The hyperfine couplings were closely resemble to those of $\text{SO}_3^{\cdot -}$ ⁸⁾ ($a^{\text{H}}=1.60$ mT and $a^{\text{N}}=1.45$ mT). The radical formed in the next stage was identified as $\text{HO}^{\cdot}$ based on the good agreement of the parameters with those reported⁹⁾ (Fig. 1-B, $a^{\text{H}}=1.48$ mT and $a^{\text{N}}=1.48$ mT). These results indicate that the production of $\text{S}_2\text{O}_3^{\cdot -}$ diminishes as the reaction proceeds. Instead of $\text{S}_2\text{O}_3^{\cdot -}$, $\text{HO}^{\cdot}$ became the predominant radical in the solution. The production of $\text{HO}^{\cdot}$ probably started early, because the absorption of *p*-nitrosodimethylaniline at 438 nm was decreased by addition of the freshly prepared $\text{Na}_2\text{S}_2\text{O}_3$ - H_2O_2 solution. On the other hand, DMPO spin adduct of $\text{SO}_3^{\cdot -}$ formed during photolysis of Na_2SO_3 was detectable by ESR, whereas $\text{HO}^{\cdot}$ was not detectable.⁸⁾

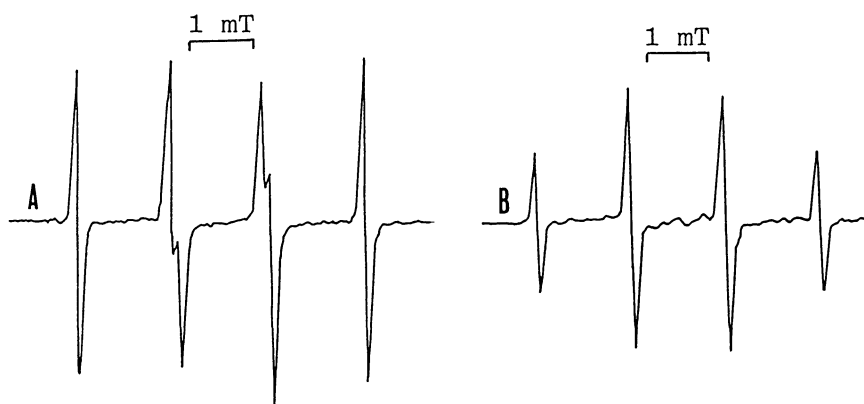


Fig. 1. The ESR spectra of DMPO-trapped radicals produced in the $\text{Na}_2\text{S}_2\text{O}_3$ - H_2O_2 system. The spectrum was recorded 2 min after the start of incubation of 34 mM $\text{Na}_2\text{S}_2\text{O}_3$, 100 mM H_2O_2 , and 200 mM DMPO at room temperature. DMPO was added 1 min (line A) or 3 h (line B) after mixing of $\text{Na}_2\text{S}_2\text{O}_3$ and H_2O_2 .

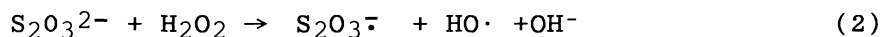
Spectral data (UV λ_{max} 245, 293 nm, EI-MS TMS deriv. M^+ m/z 643) of the product were in agreement with those of authentic 8-OH-dG.⁴⁾ Table 1 shows that 8-OH-dG was formed from dG in the $\text{Na}_2\text{S}_2\text{O}_3\text{-H}_2\text{O}_2$ system. When iron (II) ion was added to the solution, a remarkable increase of 8-OH-dG formation was observed. However, the $\text{Fe}^{2+}\text{-H}_2\text{O}_2$ system (Table 1, E,F,G) gave only trace amounts of 8-OH-dG. The molar concentration ratio of $\text{Na}_2\text{S}_2\text{O}_3\text{:H}_2\text{O}_2$ 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-dG¹¹⁾ depended on temperature, time and concentrations of dG, $\text{Na}_2\text{S}_2\text{O}_3$ 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 $\text{Na}_2\text{S}_2\text{O}_3\text{-H}_2\text{O}_2$ systems^{a)}

Entry	Reaction conditions	Yield / %
A	$\text{Na}_2\text{S}_2\text{O}_3 + \text{H}_2\text{O}_2$	0.6
B	$\text{Na}_2\text{S}_2\text{O}_3 + \text{H}_2\text{O}_2 + 2\mu\text{M FeSO}_4$	2.1
C	$\text{Na}_2\text{S}_2\text{O}_3 + \text{H}_2\text{O}_2 + 4\mu\text{M FeSO}_4$	2.5
D	$\text{Na}_2\text{S}_2\text{O}_3 + \text{H}_2\text{O}_2 + 6\mu\text{M FeSO}_4$	2.6
E	$\text{H}_2\text{O}_2 + 2\mu\text{M FeSO}_4$	0.002
F	$\text{H}_2\text{O}_2 + 4\mu\text{M FeSO}_4$	0.004
G	$\text{H}_2\text{O}_2 + 6\mu\text{M FeSO}_4$	0.007
H	$\text{Na}_2\text{S}_2\text{O}_3$	<0.001

a) Each reaction was performed in an aqueous solution containing 4 mM dG. Concentrations of $\text{Na}_2\text{S}_2\text{O}_3$ and H_2O_2 were 34 mM and 100 mM, respectively.

Thiosulfate radical anion may arise in the $\text{Na}_2\text{S}_2\text{O}_3\text{-H}_2\text{O}_2$ reaction as follows.⁶⁾

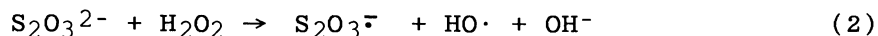


Sulfur trioxide radical anion may arise from sulfite in the similar manner.^{6,12)} Although sodium sulfite solution (8 mM) converted dG to 8-OH-dG (yield 0.5% at 37 °C for 24 h, in the dark), the formation of 8-OH-dG was suppressed (yield 0.03%) when hydrogen peroxide was present in the solution. These results indicate that the $\text{Na}_2\text{SO}_3\text{-H}_2\text{O}_2$ system provides less $\text{HO}\cdot$ generation. So the $\text{Na}_2\text{S}_2\text{O}_3\text{-H}_2\text{O}_2$ system is a unique system generating hydroxyl radical. However, equation (2) does not explain the prolonged formation of hydroxyl radical. It is considered that $\text{Na}_2\text{S}_2\text{O}_3$ generates a

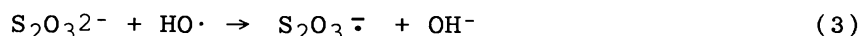
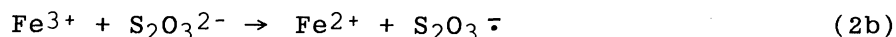
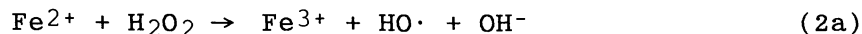
chemical species which accelerates the Fenton reaction. When $\text{Na}_2\text{S}_2\text{O}_3$ was titrated with H_2O_2 , pH of the solution drastically changed: neutral $\rightarrow$ alkaline $\rightarrow$ neutral $\rightarrow$ acidic. The end point expressed as a molar ratio of $\text{Na}_2\text{S}_2\text{O}_3:\text{H}_2\text{O}_2$ was 1:4.

We propose a reaction mechanism to elucidate our results.

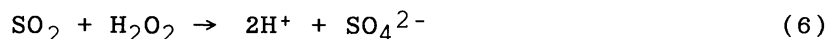
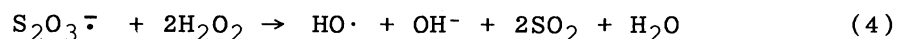
The first stage (from neutral to alkaline):



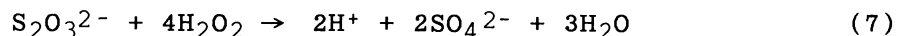
This reaction involves catalytic effect of iron ion:



The second stage (from alkaline to acidic):



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



Sodium thiosulfate is administered as an antidote to victims of arsenic or mercury poisoning. The present results imply that usage of $\text{Na}_2\text{S}_2\text{O}_3$ for chemotherapy may lead to generation of S_2O_3^- and $\text{HO}\cdot$ radicals *in vivo*, because the co-factors, iron ion and H_2O_2 , normally exist in human organs.

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- 10) 3,3'-[3,3'-Dimethyl-(1,1'-biphenyl)-4,4'-diyl]-bis[2-(p-nitrophenyl)-5-phenyl-2H 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%).
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