Free Rad. Res., Vol. 27(4), pp. 409–418
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DNA Single Strand Breaks by Aromatic Nitroso Compounds in the Presence of Thiols

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Accepted by Prof. E. Niki

(Received 13 January 1997; In revised form 19 May 1997)

Aromatic nitroso compounds, nitrosobenzene (NB), *N,N*-dimethyl-4-nitrosoaniline (DMNA) and 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS), caused DNA single strand breaks in the presence of thiol compounds. The strand breaking was inhibited completely by free radical scavenger ethanol. Electron spin resonance (ESR) studies showed that hydronitroxyl (or sulfur-substituted nitroxyl) radicals were generated in the early stage of the interactions. Formation of these radicals was not inhibited by ethanol, indicating that these radicals did not directly contribute to the strand breaking. The DNA strand breaking was inhibited partially by superoxide dismutase and catalase under the limited conditions, but not by removal of oxygen from or addition of metal chelators to the reaction mixture. By ESR-spin trapping technique using 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO), the DMPO-OH spin adduct was detected. Formation of the spin adduct was inhibited by superoxide dismutase and catalase. The hydronitroxyl (or the sulfur-substituted nitroxyl) radicals may reduce oxygen into active oxygen species and also transformed by themselves into other unidentified free radical species to cause the DNA strand breaks.

Keywords: Aromatic nitroso compound, thiol, nitroxyl radical, active oxygen species, DNA single strand break

INTRODUCTION

It is known that some aromatic amines including aniline[1] and *N,N*-dimethyl-*p*-phenylenediamine[2] act as mutagens and/or carcinogens. The aromatic amines are converted into the corresponding *N*-hydroxyamino compounds by the hydroxylating enzymes in liver.[3] *N*-Hydroxyamino compounds have been shown to damage DNA molecules by being transformed into the esters and/or the nitrenium cations that attack electrophilically the nucleobases[3] or by reducing molecular oxygen to generate active oxygen species that cleave DNA single strands.[4] On the other hand, *N*-hydroxyamino compounds are oxidized into aromatic nitroso compounds.[5] Some of the aromatic nitroso compounds show mutagenicity.[6] The reactivity of aromatic nitroso compounds, however, has not yet been well characterized. It has been suggested that aromatic nitroso compounds are converted by one electron reduction into the corresponding aromatic hydro-

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nitroxyl radicals by hemolysate or nonemzymatically by superoxide anion, NADH or NADPH, and the radicals convert molecular oxygen into active oxygen species.[5,7] In our previous investigation, it has been shown that nitrosophenols are converted into the phenoxyl radicals in the absence and presence of cysteine, and the radicals reduce molecular oxygen into active oxygen species.[8]

In the present study, it was found that aromatic nitroso compounds, nitrosobenzene (NB), *N,N*-dimethyl-4-nitrosoaniline (DMNA) and 3,5-dibromo-4-nitrosobenzene sulfonate (DBNBS) (Fig. 1), cleaved DNA single strands in the presence of thiols. These nitroso compounds generated the hydronitroxyl (or the sulfur-substituted nitroxyl) radicals by interaction with thiols which may generate active oxygen species in the presence of oxygen and other unidentified free radical species in the absence of oxygen to cause DNA strand breaking.

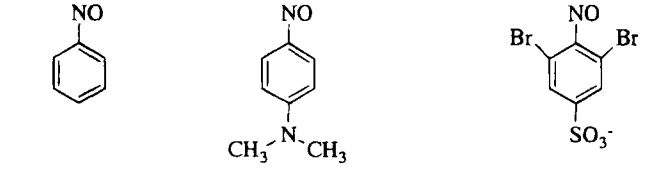
MATERIALS AND METHODS

Materials

Nitrosobenzene (NB) and *N,N*-dimethyl-*p*-nitrosoaniline (DMNA) were obtained from Tokyo Chemical Industry (Tokyo, Japan). 3,5-Dibromo-4-nitrosobenzene sulfonate (DBNBS) and 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) were obtained from Labotec Company (Tokyo, Japan). Cysteine (CysSH) was from Nacalai Tesque (Kyoto, Japan). Glutathione (GSH), metallothionein, catalase [EC 1.15.1.6] (from bovine liver 20000 U/mg protein) and superoxide dismutase (SOD) [EC 1.15.1.1] (from bovine erythrocytes 4000 U/mg protein) were obtained from Sigma Chemical Company (St. Louis, MO, USA). Plasmid pBR 322 DNA (1.0 mg/ml in 10 mM Tris-HCl (pH 8.0) and 1 mM ethylenediaminetetraacetic acid (EDTA)) was a product of New England Biolabs (Beverly, MA, USA). Other reagents were obtained from Wako Pure Chemical Industries (Osaka, Japan).

DNA Single Strand Breaks Caused by the Interaction of the Aromatic Nitroso Compounds and the Thiols

DNA strand breaking activity of aromatic nitroso compounds was examined according to the method previously described.[9,10] A 5-µl aliquot of the solution of supercoiled pBR 322 DNA in 0.1 M phosphate buffer (pH 7.4) at the final concentration of 10 µg/ml was mixed with 5 µl of a solution of the aromatic nitroso compound at the final concentration of 10 mM, the thiol compound and the active oxygen scavenger in 0.1 M phosphate buffer (pH 7.4) in a plastic tube with a stopper, and the mixture was incubated at 37°C for the indicated period. After addition of 1 µl of a 0.5% bromophenol blue/0.5% xylene cyanol/50% glycerol solution, the whole mixture was subjected to agarose gel electrophoresis. Agarose gel electrophoresis was run at 4 V/cm for 2 h using a Mupid-2 submarine electrophoretic apparatus (Advance Company, Tokyo, Japan).



Nitrosobenzene (NB) | *N,N*-Dimethyl-4-nitrosoaniline (DMNA) | 3,5-Dibromo-4-nitrosobenzene sulfonate (DBNBS)

FIGURE 1 Structures of aromatic nitroso compounds examined in the present study.

The buffer used for the electrophoresis contained 45 mM Tris-borate buffer (pH 8.3), 1 mM EDTA-Na_2 and 0.5 μg/ml ethidium bromide. The gel had been prepared by dissolving 0.7% SeaKem ME agarose (FMC BioProducts, Rockland, ME, USA) in the buffer. The band was visualized by irradiation at 300 nm.

Electron Spin Resonance (ESR) Spectra

ESR spectra were obtained on a X-band JES-RE1X spectrometer (JEOL, Tokyo, Japan) with a Mn^{2+} marker using a capillary tube or a flat quartz cell at room temperature. Operating conditions were: field setting at 336.0 mT, scanning range of 10 mT, modulation frequency at 100 kHz, microwave power at 10 mW and modulation amplitude at 0.1 mT.

RESULTS

It is known that a supercoiled (form I) DNA is convertible into a nicked open circular form (form II) DNA and/or into a linear form (form III) DNA when its single strands are cleaved, and they can be separately detected on agarose gel electrophoresis.[9] When supercoiled plasmid pBR 322 DNA was incubated with 5 mM NB at pH 7.4 and 37°C overnight, supercoiled form I DNA was unchanged (Fig. 2, lane 2). In contrast, when the DNA was incubated with a mixture of NB and 10 mM CysSH for 5 h, a significant amount of form II DNA appeared, indicating that the single strands were cleaved (lane 3). The breaking was inhibited in the presence of a mixture of SOD and catalase (lane 4). The breaking by incubating overnight (lane 5) was not inhibited in the presence of a mixture of SOD and catalase, by removal of oxygen from the reaction mixture, or by addition of 50 mM EDTA or diethylenetriaminepentaacetic acid (DTPA) (data not shown), but inhibited completely in the presence of ethanol (lane 6). When supercoiled DNA was incubated with a mixture of NB and 10 mM GSH for 5 h, single strand breaks were caused (lane 7). The breaking was inhibited in the presence of a mixture of SOD and catalase (lane 8). The breaking by incubating overnight (lane 9) was not inhibited in the presence of a mixture of SOD and catalase or by removal of oxygen from the reaction mixture (data not shown), but inhibited completely in the presence of ethanol (lane 10). When supercoiled DNA was incubated with a mixture of NB and metallothionein overnight, single strand breaks were caused (lane 11).

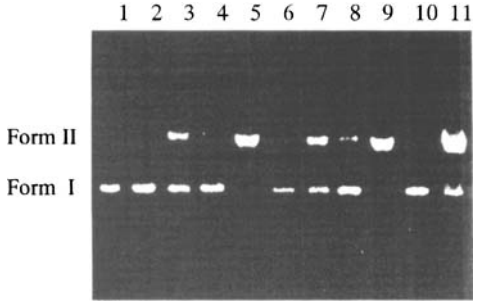


FIGURE 2 Agarose gel electrophoresis of the incubation mixture of supercoiled DNA and NB. A solution of supercoiled pBR 322 DNA (10 μg/ml) in 0.1 M phosphate buffer (pH 7.4) was incubated at 37°C with the following compound at the indicated period. Lane 1, none overnight; lane 2, 5 mM NB overnight; lane 3, 5 mM NB/10 mM CysSH for 5 h; lane 4, 5 mM NB/10 mM CysSH/1 mg/ml SOD + 1 mg/ml catalase for 5 h; lane 5, 5 mM NB/10 mM CysSH overnight; lane 6, 5 mM NB/10 mM CysSH/10% ethanol overnight; lane 7, 5 mM NB/10 mM GSH for 5 h; lane 8, 5 mM NB/10 mM GSH/1 mg/ml SOD + 1 mg/ml catalase for 5 h; lane 9, 5 mM NB/10 mM GSH overnight; lane 10, 5 mM NB/10 mM GSH/10% ethanol overnight; and lane 11, 5 mM NB/17 mg/ml metallothionein overnight. The mixture was subjected to agarose gel electrophoresis. The electrophoretic positions of supercoiled DNA (form I) and nicked open circular DNA (form II) are indicated.

When supercoiled DNA was incubated with 5 mM DMNA at pH 7.4 and 37°C overnight, supercoiled form I DNA was slightly changed into form II DNA (Fig. 3, lane 2). When the DNA was incubated with a mixture of DMNA and 10 mM CysSH for 5 h, single strands were cleaved (lane 3). The breaking was inhibited in the presence of a mixture of SOD and catalase (lane 4). The extensive breaking induced by incubating overnight (lane 5) was not inhibited in the presence of a mixture of SOD and catalase, by

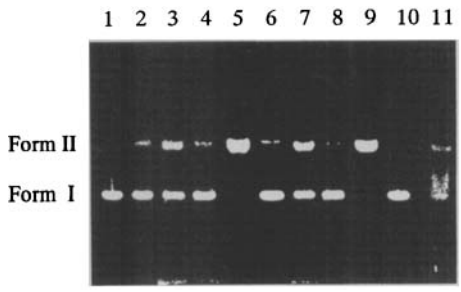


FIGURE 3 Agarose gel electrophoresis of the incubation mixture of supercoiled DNA and DMNA. A solution of supercoiled pBR 322 DNA (10 μg/ml) in 0.1 M phosphate buffer (pH 7.4) was incubated at 37 °C with the following compound at the indicated period. Lane 1, none overnight; lane 2, 5 mM DMNA overnight; lane 3, 5 mM DMNA/10 mM CysSH for 5 h; lane 4, 5 mM DMNA/10 mM CysSH/1 mg/ml SOD + 1 mg/ml catalase for 5 h; lane 5, 5 mM DMNA/10 mM CysSH overnight; lane 6, 5 mM DMNA/10 mM CysSH/10% ethanol overnight; lane 7, 5 mM DMNA/10 mM GSH for 5 h; lane 8, 5 mM DMNA/10 mM GSH/1 mg/ml SOD + 1 mg/ml catalase for 5 h; lane 9, 5 mM DMNA/10 mM GSH overnight; lane 10, 5 mM DMNA/10 mM GSH/10% ethanol overnight; and lane 11, 5 mM DMNA/17 mg/ml metallothionein overnight. The mixture was subjected to agarose gel electrophoresis. The electrophoretic positions of supercoiled DNA (form I) and nicked open circular DNA (form II) are indicated.

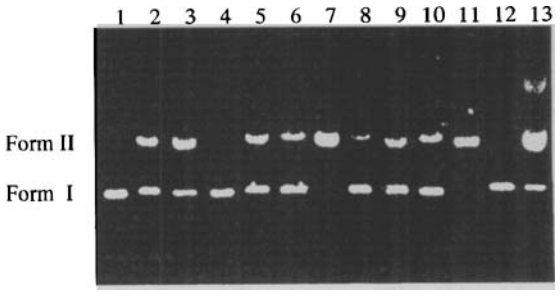


FIGURE 4 Agarose gel electrophoresis of the incubation mixture of supercoiled DNA and DBNBS. A solution of supercoiled pBR 322 DNA (10 μg/ml) in 0.1 M phosphate buffer (pH 7.4) was incubated at 37 °C with the following compound at the indicated period. Lane 1, none overnight; lane 2, 0.1 M DBNBS overnight; lane 3, 0.1 M DBNBS/1 mg/ml SOD + 1 mg/ml catalase overnight; lane 4, 0.1 M DBNBS/10% ethanol overnight; lane 5, 0.1 M DBNBS/10 mM CysSH for 1 h; lane 6, 0.1 M DBNBS/10 mM CysSH/1 mg/ml SOD + 1 mg/ml catalase for 1 h; lane 7, 0.1 M DBNBS/10 mM CysSH overnight; lane 8, 0.1 M DBNBS/10 mM CysSH/10% ethanol overnight; lane 9, 0.1 M DBNBS/10 mM GSH for 1 h; lane 10, 0.1 M DBNBS/10 mM GSH/1 mg/ml SOD + 1 mg/ml catalase for 1 h; lane 11, 0.1 M DBNBS/10 mM GSH overnight; lane 12, 0.1 M DBNBS/10 mM GSH/10% ethanol overnight; and lane 13, 0.1 M DBNBS/17 mg/ml metallothionein overnight. The mixture was subjected to agarose gel electrophoresis. The electrophoretic positions of supercoiled DNA (form I) and nicked open circular DNA (form II) are indicated.

removal of oxygen from the reaction mixture or by addition of EDTA or DTPA (data not shown), but inhibited completely in the presence of ethanol (lane 6). When supercoiled DNA was incubated with a mixture of DMNA and 10 mM GSH for 5 h (lane 7), single strands were cleaved, and the breaking was inhibited in the presence of a mixture of SOD and catalase (lane 8). The breaking by incubation overnight (lane 9) was not inhibited by removal of oxygen from the reaction mixture (data not shown), but inhibited completely by ethanol (lane 10). When supercoiled DNA was incubated with a mixture of DMNA and metallothionein overnight, extensive single strand breaks were caused (lane 11).

When supercoiled DNA was incubated with 0.1 M DBNBS at pH 7.4 and 37 °C overnight, single strand breaks were induced (Fig. 4, lane 2). The breaking was not inhibited in the presence of a mixture of SOD and catalase (lane 3), but inhibited completely in the presence of ethanol (lane 4). The results were consistent with those obtained previously.[9] When supercoiled DNA was incubated with a mixture of DBNBS and 10 mM CysSH for 1 h (lane 5) or overnight (lane 7), single strand breaks were enhanced. The breaking was not inhibited in the presence of a mixture of SOD and catalase (lane 6), but inhibited in the presence of ethanol (lane 8). When supercoiled DNA was incubated with a mixture of DBNBS and 10 mM GSH for 1 h (lane 9) or overnight (lane 11), single strand breaks were enhanced, and the breaking was not inhibited in the presence of a mixture of SOD and catalase (lane 10), but inhibited completely in the presence of ethanol (lane 12). When supercoiled DNA was incubated with a mixture of DBNBS and metallothionein, extensive single strand breaks were caused (lane 13). These results indicate that DBNBS by itself has the potency to cause DNA single strand breaks as has been previously reported,[11] and the activity was enhanced by the thiols.

The results obtained above indicated that aromatic nitroso compounds effectively cleaved DNA single strands. The breaking was partially inhibited by a mixture of SOD and catalase, indicating a partial contribution of active oxygen species in the breaking. However, the breaking may be caused by an alternative pathway via other reactive species because the breaking was not prevented by removal of oxygen from the reaction mixture and by addition of EDTA and DTPA.

In order to characterize the radical species generated in the interaction of the aromatic nitroso compounds with the thiols, ESR studies were performed. ESR spectra of a mixture of NB and CysSH (or GSH) incubated at pH 7.4 and room temperature for 0–3 h were recorded. Characteristic multi-line ESR signals appeared after 5-min incubation of a mixture of NB and CysSH (Fig. 5, A) and a mixture of NB and GSH (Fig. 5, B), and the signals gradually disappeared after 3-h incubation. The multi-line ESR signals were consistent with those of the computer-simulated ESR signals (Fig. 5, C) based on the corresponding hydronitroxyl radical (Fig. 5, D) with the indicated hyperfine splitting constants (hfsc), which are similar to those of the same radical reported earlier: a_N = 0.91, a_H^{NH} = 1.19, $a_H o,o,p$ = 0.30 and $a_H m$= 0.14 mT.[12]

ESR spectra of a mixture of DMNA and CysSH (or GSH) incubated at pH 7.4 and room temperature for 0–3 h were recorded. Characteristic multi-line ESR signals appeared after 5-min incubation and the intensities of the signals were maximal after 3-h incubation of the mixture of DMNA and CysSH (Fig. 6, A) and the mixture of DMNA and GSH (Fig. 6, B). The multi-line ESR signals were consistent with those of the computer-simulated ESR signals (Fig. 6, C) based on the corresponding hydronitroxyl radical (Fig. 6, D) with the indicated hfsc. In these spectra, unidentified broad signals overlapped to the multi-line signals were observed, indicating the formation of other radical species.

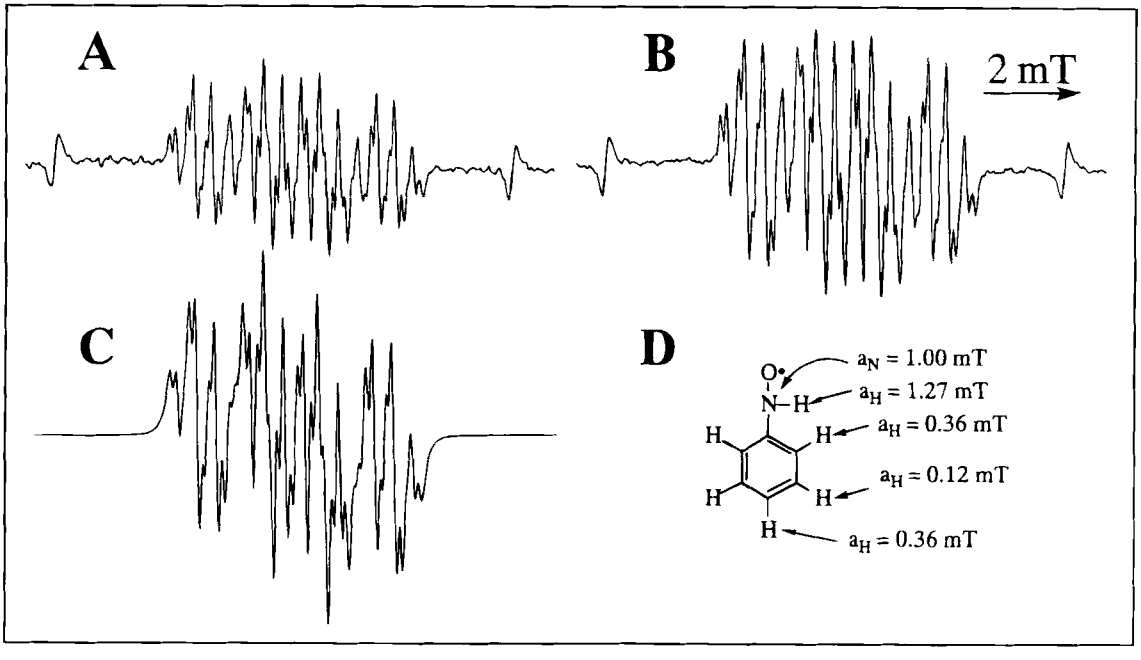


FIGURE 5 ESR spectra of the mixture of 5 mM NB/10 mM CysSH (**A**) and 5 mM NB/1 mM GSH (**B**) in 0.1 M phosphate buffer (pH 7.4) incubated at room temperature for 5 min, and the computer-simulation (**C**) of the benzene hydronitroxyl radical (**D**) with the indicated hfsc. In A and B, receiver gain was set at 5000, and the *g*-values of the signals of Mn^{2+} marker seen in the extremely left and right sides were 2.034 and 1.981, respectively.

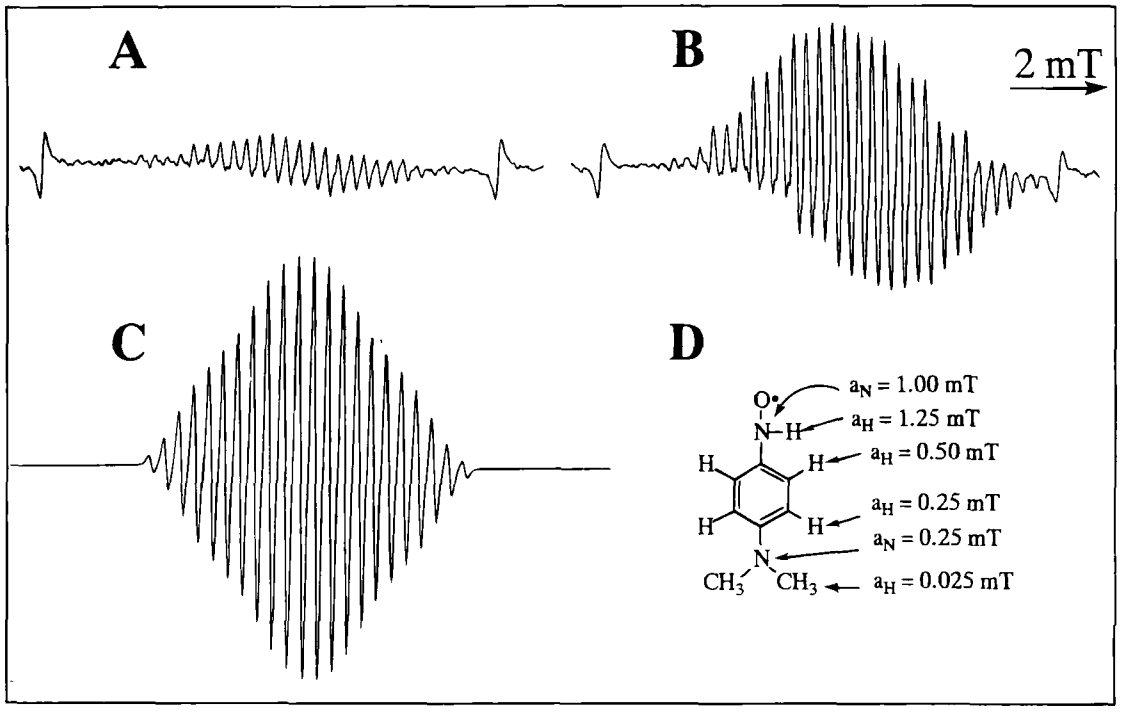


FIGURE 6 ESR spectra of the mixture of 5 mM DMNA/10 mM CysSH (**A**) and 5 mM DMNA/10 mM GSH (**B**) in 0.1 M phosphate buffer (pH 7.4) incubated at room temperature for 3 h, and the computer-simulation (**C**) of the *p*-(dimethylamino)benzene hydronitroxyl radical (**D**) with the indicated hfsc. In A and B, receiver gain was set at 5000, and the *g*-values of the signals of Mn^{2+} marker seen in the extremely left and right sides were 2.034 and 1.981, respectively.

ESR spectra of a mixture of 0.1 M DBNBS and 10 mM CysSH (or GSH) incubated at pH 7.4 and room temperature for 0–10 h were recorded. After 5-min incubation, unidentified complex ESR signals appeared, and after 5-h incubation characteristic 3-line signals with hfsc of a_N = 1.29 mT appeared, which was ascribable to a nitroxyl radical formed from sulfur-containing radical[13,14] (data not shown). Two kinds of radicals were generated in the interaction of DBNBS with the thiols. The results were consistent with those of the earlier studies.[11]

It was found that the generation of these hydronitroxyl and nitroxyl radicals could not be prevented in the presence of ethanol (data not shown), indicating that these radicals did not directly participate in the DNA single strand breaks, because the DNA single strand breaks were effectively inhibited in the presence of ethanol.

In order to characterize unstable radical species generated in the interaction of the aromatic nitroso compounds with the thiols, ESR-spin trapping technique was employed by using spin trapping agent DMPO. ESR spectrum of a mixture of DMPO, NB and CysSH incubated at pH 7.4 and room temperature for 5 min showed the ESR signals (Fig. 7, A) composed of 4-line 1:2:2:1 signals of characteristic DMPO-OH spin adduct (Fig. 7, E) with hfsc of $a_N = a_H$ = 1.49 mT[15,16] and 6-line signals of characteristic DMPO-SCys spin adduct (Fig. 7, F) with hfsc of a_N = 1.52 and a_H = 1.70 mT.[17,18] The profile of the ESR signals (Fig. 7, A) was consistent with that of the computer-simulated ESR signals (Fig. 7, C) of a mixed DMPO-OH spin adduct (Fig. 7, E) and DMPO-SCys spin adduct (Fig. 7, F). Both hydroxyl and CysSH thiyl radicals were generated in the interaction of NB with CysSH. ESR spectrum of a mix-

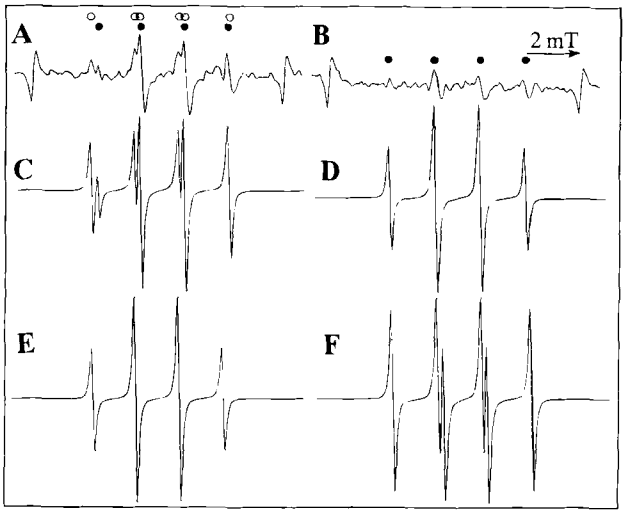


FIGURE 7 ESR spectra of the mixture of 0.1 M DMPO/5 mM NB/ 0.1 M CysSH (**A**), 0.1 M DMPO/5 mM NB/10 mM GSH (**B**) in 0.1 M phosphate buffer (pH 7.4) incubated at room temperature for 5 min, and the computer-simulation of the mixed DMPO-OH and DMPO-SCys spin adducts (**C**), DMPO-SG spin adduct with hfsc of a_N = 1.54 mT and a_H = 1.58 mT (Ref. 13,14) (**D**), DMPO-OH adduct with hfsc of $a_N = a_H$ = 1.49 mT (Ref. 11,12) (**E**) and DMPO-SCys adduct with hfsc of a_N = 1.52 mT and a_H = 1.70 mT (Ref. 13,14) (**F**).

ture of DMPO, NB and GSH incubated at pH 7.4 and room temperature for 5 min showed the 4-line ESR signals characteristic to those of DMPO-SG spin adduct (Fig. 7, B) with hfsc of a_N = 1.54 and a_H = 1.58 mT.[18,19] The profile of the ESR signals was consistent with that of the computer-simulated ESR signals (Fig. 7, D) of DMPO-SG spin adduct. It was suggested that GSH thiyl radical was generated in the interaction of NB with GSH.

When the spin adduct formation was performed in the presence of a mixture of SOD and catalase, both the signals due to DMPO-OH and DMPO-SCys did not appear (Fig. 8, A). ESR spectrum of a mixture of DMPO, DMNA and CysSH incubated at pH 7.4 and room temperature for 5 min showed the ESR signals (Fig. 8, B) composed of 4-line 1:2:2:1 signals of characteristic DMPO-OH spin adduct and 6-line signals of characteristic DMPO-SCys spin adduct appeared, and the signals did not appear when the reaction was conducted in the presence of a mixture of SOD and catalase. ESR spectrum of a mixture of DMPO, DBNBS and CysSH incubated at pH 7.4 and room temperature for 5 min showed the ESR signals (Fig. 8, C) com-

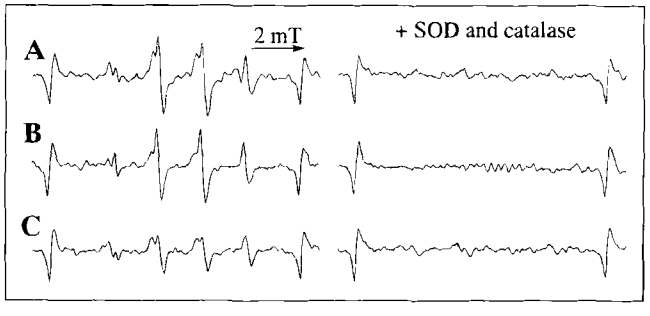


FIGURE 8 ESR spectra of 0.1 M DMPO/5 mM NB/0.1 M CysSH (**A**), 0.1 M DMPO/5 mM DMNA/0.1 M CysSH (**B**) and 0.1 M DMPO/5 mM DBNBS/0.1 M CysSH (**C**) in 0.1 M phosphate buffer (pH 7.4) incubated in the absence and the presence of 0.1 mg/ml SOD and 0.1 mg/ml catalase at room temperature for 5 min.

posed of 4-line 1:2:2:1 signals of characteristic DMPO-OH spin adduct and 6-line signals of characteristic DMPO-SCys spin adduct appeared, and the signals did not appear when the reaction was conducted in the presence of a mixture of SOD and catalase. Hence, hydroxyl and CysSH thiyl radicals were generated in the interaction of these aromatic nitroso compounds with CysSH, and these radicals may be derived from superoxide and/or hydrogen peroxide.

DISCUSSION

Aromatic nitroso compounds, NB, DMNA and DBNBS cleaved DNA single strands in the presence of CysSH, GSH and metallothionein. The breaking was effectively inhibited by ethanol, a scavenger of free radicals. It was found by the ESR studies that the hydronitroxyl radicals were generated in the early stage of the interaction of NB and DMNA with the thiols, and unidentified radical(s) and the sulfur-substituted nitroxyl radical were generated in the early stage of the interaction of DBNBS and the thiols. These characteristic radicals may not, however, participate directly in the DNA strand cleavage, because the formation of these radicals could not be prevented by ethanol.

Active oxygen species participated partially in the breaking because the breaking was prevented by SOD and catalase under certain limited conditions. Superoxide and/or hydrogen peroxide-dependent hydroxyl radical generation in the mixtures of the aromatic nitroso compounds and the thiols was demonstrated by the ESR-spin trapping technique using DMPO. The DMPO-OH spin adduct was detected in the mixtures and its formation was prevented in the presence of SOD and catalase. Superoxide can be transformed into hydrogen peroxide, which is in turn converted into strongly reactive hydroxyl radical. It is well-known that hydroxyl radical effectively cleaves DNA single strands.[19–21] Hence, the participation of hydroxyl radical in the DNA strand breaking by the aromatic nitroso compounds and the thiols is indicated.

In the reaction mixtures of the aromatic nitroso compounds and the thiols, the DMPO-SR (R = Cys or G) spin adducts were formed together with the DMPO-OH spin adduct. The formation of the spin adducts was dependent on superoxide and/or hydrogen peroxide. The DMPO-SR spin adduct may be formed by the following reactions (Fig. 9). In one way the thiyl radicals may be generated by the interaction of hydroxyl radical and the thiols, and both hydroxyl radical and the thiyl radicals may be trapped as the DMPO-OH and the DMPO-SR spin adducts. In another way, the DMPO-OH spin adduct may react with the thiols to yield ESR-silent DMPO-OH_2 and the thiyl radicals, the latter being trapped by an excess amount of DMPO as the DMPO-SR spin adduct.[22] Because the formation of the DMPO-SR adducts is possibly due to the reaction of the DMPO-OH spin adduct with the thiols, formation of the thiyl radicals in the reaction of hydroxyl radical and the thiols could not be clearly demonstrated in the present study. Hence, the contribution of the thiyl radicals in the DNA strand breaking cannot be evaluated.

Active species in the interaction of the aromatic nitroso compounds with the thiols to cause the DNA single strand breaking could not be always ascribable to active oxygen species. The DNA strand breaking could not be prevented by removal of oxygen from the reaction mixtures, and also by addition of a metal chelator that prevent metal-induced superoxide and/or hydrogen peroxide-dependent production of hydroxyl radical. Because ethanol, a scavenger of free radicals, effectively prevented the strand breaking, certain unidentified free radicals other than hydronitroxyl (or sulfur-substituted nitroxyl radicals) and active oxygen species must have generated to cleave the DNA strands.

Possible mechanisms for the DNA strand breaking by the interaction of the aromatic nitroso compounds with the thiols are postulated in Fig. 10. In one way, the aromatic nitroso com-

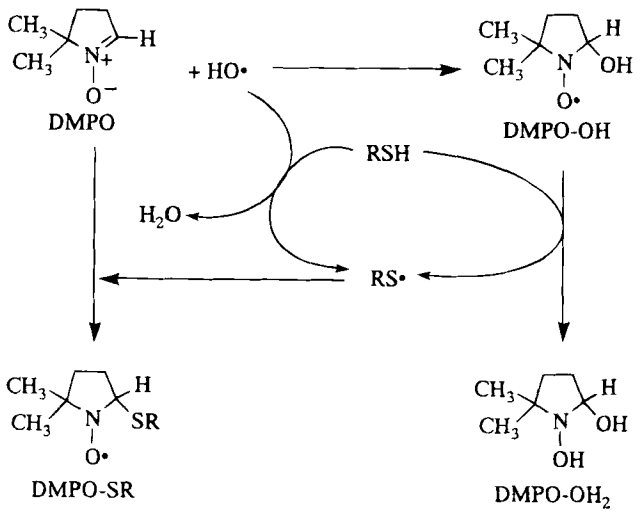


FIGURE 9 Possible mechanisms for the formation of the DMPO-OH and DMPO-SR spin adducts in the reaction of DMPO, hydroxyl radical and RSH (RSH = CysSH or GSH).

pounds may be reduced by the thiols in an early stage of the interaction into the corresponding hydronitroxyl (or sulfur-substituted nitroxyl) radicals, which in turn reduced molecular oxygen into superoxide. Superoxide may dismutate into hydrogen peroxide, and hydrogen peroxide may be converted into hydroxyl radical with an aid of a trace amount of metal ions. Hydroxyl radical may cleave DNA single strands. In another way, the hydronitroxyl (or the sulfur-substituted nitroxyl) radicals may create free radical species other than active oxygen species to cause the DNA strand breaking.

In a toxicological view point, it may be important that aromatic nitroso compounds, metabolites of aromatic amines, caused damage to the DNA molecule to induce single strand breaks by interaction of CysSH, GSH and metallothionein.

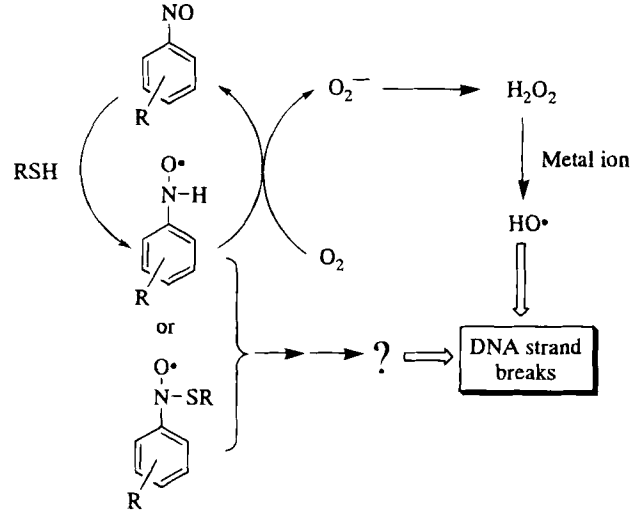


FIGURE 10 Possible mechanisms for the DNA single strand breaks by aromatic nitroso compounds in the presence of thiols.

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