

Method for assessing X-ray-induced hydroxyl radical-scavenging activity of biological compounds/materials

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A method for correctly assessing hydroxyl radical scavenging activity of antioxidative chemicals and/or biological compounds/materials was proposed. This method can simultaneously assess two factors, i.e. hydroxyl radical-scavenging and 5,5-dimethyl-2-hydroxy-1-pyrrolidine-*N*-oxide (hydroxyl radical adduct of 5,5-dimethyl-1-pyrroline-*N*-oxide)-reducing ability, as antioxidative properties. In this paper, some biologically common hydrophilic molecules, cell culture media, and rat plasma were tested. X-ray-induced hydroxyl radical can be detected using the electron paramagnetic resonance spin trapping technique. Using X-ray irradiation of the reaction mixture as the hydroxyl radical source, the true hydroxyl radical-scavenging ability of the subjected antioxidant can be assessed. In addition, the method simultaneously measures the reduction of 5,5-dimethyl-2-hydroxy-1-pyrrolidine-*N*-oxide, to estimate the reducing ability of the antioxidant. Biological materials, such as sugars and proteins, could abolish hydroxyl radical at the biological concentration. Ascorbic acid showed reducing ability at the biological concentration. The simultaneous assessment of hydroxyl radical-scavenging and reducing ability of antioxidants can be an informative index for antioxidants.

Key Words: hydroxyl radical, antioxidant, X-ray, electron paramagnetic resonance, spin trapping

Hydroxyl radical ($\cdot\text{OH}$), which is the most reactive among the reactive oxygen species (ROS), can be measured using the electron paramagnetic resonance (EPR) spin-trapping method.⁽¹⁻⁵⁾ Spin-trapping agents can react with short-lived free radicals, such as $\cdot\text{OH}$, superoxide ($\text{O}_2^{\cdot-}$) and other organic radicals to make a relatively stable nitroxyl radical form, the so-called spin adduct. 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) is probably the most common spin-trapping agent.⁽⁶⁾ The profile of the EPR spectrum of the spin adduct is characterized by the free radical species. The EPR spectrum of the $\cdot\text{OH}$ -adduct of DMPO (DMPO-OH) gives 4 characteristic lines with an intensity ratio of 1:2:2:1. The $\text{O}_2^{\cdot-}$ -adduct of DMPO (DMPO-OOH) gives a slightly complicated EPR spectrum with 12 lines.

$\cdot\text{OH}$ is an important player in biological oxidative stress due to its high reactivity. Ability to remove $\cdot\text{OH}$ is therefore an important factor for evaluating the effectiveness of an antioxidant. The $\cdot\text{OH}$ -scavenging ability of an antioxidant has been estimated by a method adding the antioxidant and a spin-trapping agent to a $\cdot\text{OH}$ generation system.⁽⁷⁻¹⁶⁾ The Fenton reaction,⁽⁷⁻¹¹⁾ irradiating hydrogen peroxide (H_2O_2) with UV⁽¹²⁻¹⁴⁾ or ionized radiation^(15,16) has been often used as the $\cdot\text{OH}$ source. When the Fenton reaction system is used as the $\cdot\text{OH}$ source for estimating the $\cdot\text{OH}$ -scavenging ability of an antioxidant, the iron-chelating effect of the antioxidant and/or direct consumption of H_2O_2 by the antioxidant must be considered for a correct assessment. When the

UV+ H_2O_2 reaction system is used as the $\cdot\text{OH}$ source, similarly, the reaction of H_2O_2 with the antioxidant, absorption of UV often by colored antioxidants, and contamination of metal ions, such as Fe^{2+} and Cu^+ , must be again considered for correct assessment of the $\cdot\text{OH}$ -scavenging ability. Otherwise, it will be confusing whether the generated $\cdot\text{OH}$ was scavenged or generation of $\cdot\text{OH}$ was inhibited. For example, Matsui *et al.*⁽¹⁷⁾ reported that astaxanthin, curcumin and rutin eliminated DMPO-OH in a dose-dependent manner when the Fenton reaction or photolysis of H_2O_2 was used as the source of $\cdot\text{OH}$; however, astaxanthin, curcumin and rutin could not eliminate DMPO-OH generation by the X-ray irradiation of water. Yoshioka *et al.*⁽¹⁶⁾ also mentioned that antioxidants disrupts the chemical system for generating $\cdot\text{OH}$; therefore, gamma irradiation was used as the $\cdot\text{OH}$ source for their experiment.

X-ray irradiation of water can also generate $\cdot\text{OH}$ by direct ionization of water molecules. Using this simple system, estimation of the $\cdot\text{OH}$ -scavenging-ability of an antioxidant is much easier, because chemical inhibition of $\cdot\text{OH}$ generation does not have to be considered. In addition, $\cdot\text{OH}$ generation does not continue after X-ray irradiation has stopped. Nonetheless, reduction of the spin adduct by the subjected antioxidant must be considered to avoid a misleading result. The reduction of the spin adduct by the subjected antioxidant can be easily estimated from the time course of the EPR measurement after irradiation. In this study, an efficient method for estimating the $\cdot\text{OH}$ -scavenging ability of a water-soluble chemical and/or biological compound was proposed using X-ray as the $\cdot\text{OH}$ source. The $\cdot\text{OH}$ -scavenging abilities of several compounds were tested, and then the method was applied to biological samples.

Materials and Methods

Chemicals. DMPO was purchased from LABOTEC Co. (Tokyo, Japan). Other chemicals were of analytical grade. As the basic solvent of reaction mixtures, 100 mM phosphate buffer (PB) (pH 7.0) containing 0.05 mM diethylenetriaminepentaacetic acid (DTPA) (100 mM PB) was prepared and used for all experiments. Deionized water (deionization by the Milli-Q system) was used for preparing 100 mM PB.

Estimating Intact DMPO-OH generation during X-ray irradiation. A reaction mixture containing 15 mM DMPO was prepared using 100 mM PB. The reaction mixture was irradiated by 32 Gy X-ray. The details of X-ray irradiation are described below. Time course of the EPR spectrum of DMPO-OH for 10 min was measured using an X-band EPR spectrometer immediately

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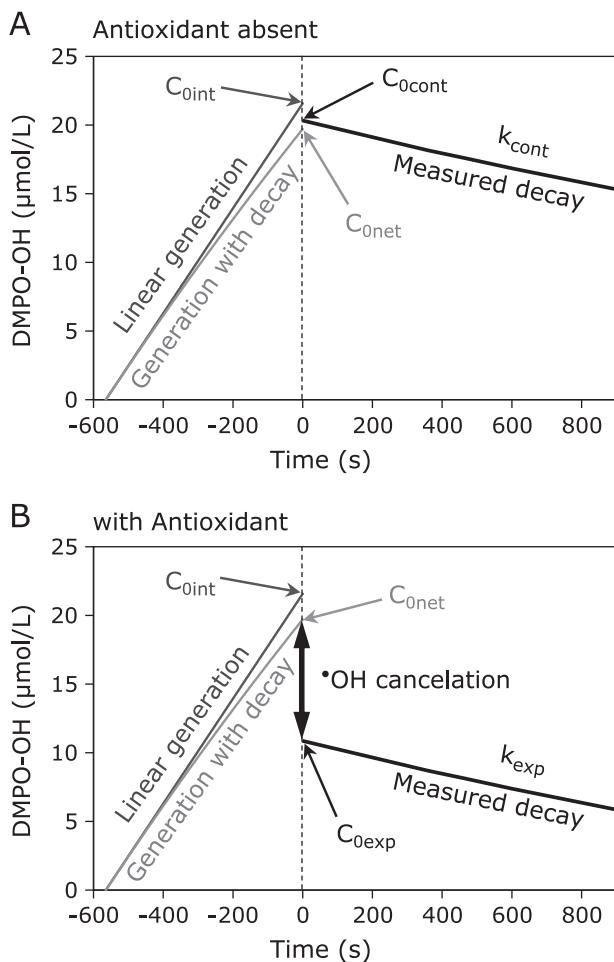


Fig. 1. Relation between experimentally measured values and estimated/simulated values. (A) Estimation of intact amount of DMPO-OH, C_{0int} , generated during X-ray irradiation eliminating natural decay of DMPO-OH when antioxidant was absent. C_{0cont} was used as the initial value of C_{0int} , and then C_{0int} was adjusted to make C_{0net} equal to C_{0cont} . (B) Estimation of \cdot OH-scavenging ability of an antioxidant. C_{0net} was calculated using the C_{0int} previously obtained and the experimentally obtained k_{exp} . The \cdot OH-scavenging ability was estimated as percentage difference of C_{0exp} from C_{0net} .

after X-ray irradiation. The EPR condition is described below. Fig. 1 shows a schematic drawing of the relation between measured data and estimated values. The natural decay rate, k_{cont} , of DMPO-OH was estimated from the decay curve of DMPO-OH after irradiation (Fig. 1A). The concentration of DMPO-OH at the end of X-ray irradiation, C_{0cont} , was obtained by extrapolating the experimental decay curve of DMPO-OH to time = 0 (end of irradiation). Intact total amount of DMPO-OH during X-ray irradiation, C_{0int} , could be obtained by linear generation of DMPO-OH when the decay of DMPO-OH during X-ray irradiation was eliminated. At first, C_{0cont} was used as the initial value of C_{0int} . Then the generation of DMPO-OH during X-ray irradiation was corrected considering the natural decay of DMPO-OH with the decay rate of k_{cont} to obtain the net generation of DMPO-OH, C_{0net} . Adjusting C_{0int} to $C_{0net} = C_{0cont}$, the total amount of DMPO-OH during X-ray irradiation, C_{0int} , was finally decided.

Estimation of \cdot OH-scavenging ability of chemical and biological compounds. A reaction mixture containing 15 mM DMPO and an arbitrary concentration of a sample compound was prepared with 100 mM PB and irradiated by 32 Gy X-ray. The

reduction rate of DMPO-OH, k_{exp} , and the concentration of DMPO-OH at the end of X-ray irradiation, C_{0exp} , were obtained from the reduction curve of DMPO-OH after X-ray irradiation (Fig. 1B). The net concentration of DMPO-OH at the end of X-ray irradiation, C_{0net} , in the reaction mixture containing the sample compound was calculated using the C_{0int} decided previously and k_{exp} obtained here. The percentage difference of C_{0exp} compared to C_{0net} was estimated as \cdot OH-scavenging ability, i.e. percentage cancellation of \cdot OH. The reduction rate of DMPO-OH, k_{exp} , and the \cdot OH-scavenging ability, $(1 - C_{0exp}/C_{0net}) \times 100$, were estimated for several chemical and/or biological compounds, such as dimethyl sulfoxide (DMSO), ethanol, acetic acid, caffeine, ascorbic acid (VC), mannitol, glucose, sucrose, sodium dodecyl sulfate (SDS), tris(hydroxymethyl)aminomethane (Tris), polyethylene glycol 400 (PEG400), gelatin, and bovine serum albumin (BSA) at several concentrations (Table 1). Reduction rate of DMPO-OH, k_{exp} , at each concentration of the subjected compound was plotted versus the concentration, and then the second order rate constant, k_{2nd} , was obtained from the slope of the plot. Similarly, the concentration dependence of the \cdot OH-scavenging-ability was obtained, and then the half maximal inhibitory concentration (IC_{50}) value was estimated. X-ray irradiation and the subsequent EPR measurements were repeated 3 times for each concentration of sample solutions. Antioxidative ability of each compound was evaluated by both the k_{2nd} of DMPO-OH-reducing ability and the IC_{50} of \cdot OH-scavenging ability.

Estimation of \cdot OH-scavenging ability of cell culture medium. DMPO was directly dissolved in a cell culture medium to make 15 mM or reaction mixtures containing 15 mM DMPO were prepared with an arbitrary concentration of cell culture media diluted with 100 mM PB. The aliquot volume ($\approx 200 \mu\text{L}$) of the reaction mixture was irradiated by 32 Gy X-ray. The \cdot OH-scavenging abilities of the cell culture media were estimated by the method described above. Both the k_{2nd} of DMPO-OH-reducing ability and the IC_{50} of \cdot OH-scavenging ability were estimated for a cell culture medium. Here, Dulbecco's Modified Eagle's medium (DMEM), RPMI-1640 medium and Leibovitz's medium were tested.

Estimation of \cdot OH-scavenging ability of rat plasma. Male and female 16-week-old Wistar rats were food-deprived overnight. Rats were anesthetized by breathing isoflurane (4% for induction then 2% for maintain) in air flow (2.5 L/min). The whole blood of the rat was collected from the abdominal artery. The blood was centrifuged at 3,500 rpm ($1,000 \times g$) for 10 min, and plasma was collected into a microtube. DMPO was directly added to intact or diluted rat plasma to make a concentration of 15 mM. The rat plasma samples containing 15 mM DMPO were irradiated by 32 Gy X-ray. Both the k_{2nd} of DMPO-OH-reducing ability and the IC_{50} of \cdot OH-scavenging ability were estimated for rat plasma using the method described above.

X-ray irradiation. X-ray irradiation was performed at PANTAK 320S (Shimadzu, Kyoto, Japan). Effective energy was 80 keV under the following conditions: X-ray tube voltage was 200 kV, X-ray tube current was 20 mA, and the thickness and materials of the pre-filter were 0.5 mm copper and 0.5 mm aluminum. Dose rate of X-ray irradiation was 3.3 Gy/min when the distance between the X-ray tube and the sample was 30 cm.

X-band EPR measurement. One hundred microliters of the reaction mixture were drawn up into PTFE tubing (i.d. 0.32 ± 0.001 inches, wall 0.002 ± 0.0005 inches; ZEUS, Orangeburg, SC), placed in the TE mode cavity using a special sample holder, and measured by an X-band EPR spectrometer (JEOL, Tokyo, Japan). The second peak from the lower field of 4 lines of the DMPO-OH was recorded under the following conditions: microwave frequency: 9.45 GHz, microwave power: 2 mW, lower magnetic field: 336.1 mT, field sweep width: 1.25 mT, field sweep resolution: 1,024 points, sweep time: 15 s, time constant: 0.03 s, field modulation frequency: 100 kHz, and field modulation width: 0.063 mT.

Table 1. ·OH-scavenging and DMPO-OH-reducing ability of several chemical/biological compounds

Compound	IC ₅₀	k _{2nd}	MW	Concentration Tested
DMSO	6.9 mM (0.054%)	NE	78.13	0.2%, 0.1%, 0.05%, 0.01%
Ethanol	21.0 mM (0.097%)	NE	46.07	0.25%, 0.2%, 0.15%, 0.1%, 0.05%, 0.02%, 0.01%
Acetic acid	507.9 mM (2.905%)	NE	60.05	10.0%, 5.0%, 2.5%, 1.0%
Caffeine	2.0 mM (0.039%)	0.002 min ⁻¹ mM ⁻¹	194.19	0.1%, 0.05%, 0.02%, 0.01%
VC	NE	4.986 min ⁻¹ mM ⁻¹	176.12	40 μM, 20 μM, 10 μM, 5 μM
Mannitol	15.6 mM (0.284%)	NE	182.17	1.0%, 0.5%, 0.25%, 0.1%
Glucose	16.5 mM (0.297%)	NE	180.16	1.0%, 0.5%, 0.25%, 0.1%
Sucrose	10.6 mM (0.363%)	NE	342.3	1.0%, 0.5%, 0.25%, 0.1%
SDS	46.0 mM (1.327%)	NE	288.68	2.0%, 1.5%, 1.0%, 0.5%
Tris	24.4 mM (0.295%)	NE	121.1	5 mM, 10 mM, 25 mM, 50 mM
PEG400	3.19 mM (0.128%)	NE	400*	0.5%, 0.25%, 0.1%, 0.05%
Gelatin	1.18%	NE	—	3.5%, 2.0%, 1.0%, 0.5%
BSA	0.38 mM (3.14%)	NE	66000*	3.5%, 2.0%, 1.0%, 0.5%

MW: molecular weight. NE: The value could not be evaluated for the concentration range tested in this paper. *An approximate molecular weight was referenced as a guide.

The EPR measurements were repeated 10 times with 1 min intervals for each sample. EPR data acquisition was controlled by the WIN-RAD ESR Data Analyzer System (Radical Research Inc., Hino, Japan). The acquired EPR spectra were analyzed using an in-house line fitting program, and the Gaussian line shape was fitted. The signal height and line width of the fitted Gaussian line were measured and then EPR signal intensity was obtained as (signal height) × (line width).

Results and Discussion

The X-ray-irradiated sample solution was loaded on the X-band EPR spectrometer, and the measurement started 2.5–4.5 min after the end of irradiation. The time taken from the end of X-ray irradiation to starting EPR measurement was measured. DMPO-OH was generated in the reaction mixture containing DMPO by X-ray irradiation and gradually decreased after the end of X-ray irradiation. When antioxidant was absent from the reaction mixture, the natural decay rate of DMPO-OH was assessed by 12 experiments to be $0.019 \pm 0.0016 \text{ min}^{-1}$. The amount of DMPO-OH at the end of X-ray irradiation, $C_{0\text{cont}}$, was $19.91 \pm 0.728 \text{ μM}$ by extrapolating the decay curve to the end of X-ray irradiation ($t = 0$). With repeated simulation, $C_{0\text{int}}$ was adjusted to make simulated $C_{0\text{net}}$ equal to $C_{0\text{cont}}$. Finally, $C_{0\text{int}}$ was found to be $21.76 \pm 0.812 \text{ μM}$ under this experimental condition, i.e. concentration of DMPO was 15 mM and X-ray dose was 32 Gy. This result suggested that this method may have relatively large intrinsic uncertainty for the resulted ·OH-scavenging ability with a single experiment, since the percentage of the SD value to the average $C_{0\text{int}}$ value was 3.7%. To assess the ·OH-scavenging ability of a tested compound, therefore, the IC₅₀ value was estimated by experiments performed at several concentrations of the compound.

The result of estimating the antioxidative ability of DMSO is described for an example. When DMSO was added to the reaction mixture, the amount of X-ray-induced DMPO-OH in the reaction mixture decreased depending on the dose of DMSO (Fig. 2A). The time course of DMPO-OH after X-ray irradiation was measured for each experiment, and then the reduction rate, k_{exp} , was estimated from the slope of the semi-logarithmic decay plot of DMPO-OH. All experiments showed similar k_{exp} values independently of the DMSO concentration, even though the variability of k_{exp} was larger at a higher concentration of DMSO. The levels of k_{exp} obtained for DMSO experiments showed no difference among concentrations of DMPO (Fig. 2B), the level of which was similar to the control antioxidant-free experiment (around 0.02 min^{-1}). The result suggests that DMSO does not reduce DMPO-OH. Then, the DMPO-OH concentration at the end of X-ray irradiation,

$C_{0\text{exp}}$, i.e. Y-interception of each decay curve, was also estimated by extrapolating the decay curve to time = 0. $C_{0\text{exp}}$ values decreased depending on the DMSO concentration. The $C_{0\text{net}}$ value was obtained by simulating DMPO-OH generation during X-ray irradiation using $C_{0\text{int}} = 21.76$ previously determined and the k_{exp} value obtained by each measurement. The ·OH-scavenging ability of DMSO was estimated as the percentage difference of $C_{0\text{exp}}$ compared to $C_{0\text{net}}$. The k_{exp} , $C_{0\text{exp}}$, $C_{0\text{net}}$, and ·OH-scavenging ability values were obtained for each experiment and averaged for a concentration. Finally, the ·OH-scavenging ability of DMSO was plotted with the logarithmic value of the DMSO concentration, and the IC₅₀ value was found to be 6.86 mM (Fig. 2C). The result suggested that DMSO can directly scavenge ·OH. The reaction of ·OH and DMSO gives a methyl radical. In this experiment, the EPR signal of the DMPO-CH₃ adduct was separately obtained with the DMPO-OH signal (data not shown). DMPO stabilized the methyl radical; therefore, methyl radicals could not play extra reactions in this experimental system. The results suggested that the antioxidative ability of DMSO is due to ·OH-scavenging, but not due to reducing oxidants.

For the case of VC, the reduction rates of DMPO-OH after X-ray irradiation, k_{exp} , increased depending on the concentration of VC (Fig. 3A). The second-order rate constant calculated from the slope of Fig. 3A was $4.986 \text{ min}^{-1}\text{mM}^{-1}$. However, no ·OH-scavenging ability was obtained for VC in the range of concentration tested in this experiment (Fig. 3B). This result suggested that the antioxidative ability of VC is as a strong reductant, but not as an ·OH scavenger.

For other subjected compounds/materials, $k_{2\text{nd}}$ and IC₅₀ values of ·OH-scavenging ability were estimated in the same way. The results and concentration conditions are summarized in Table 1. Only VC showed a markedly faster reduction rate of DMPO-OH; however, other subjects showed similar slow k_{exp} to the control experiment for all concentrations tested. DMPO-OH is a relatively stable nitroxyl radical. VC, which is a strong reductant, can reduce this stable radical at a μM concentration; however the other molecules tested in this paper have no such strong reducing ability. Therefore $k_{2\text{nd}}$ values could not be calculated for them, except caffeine, which showed slight reducing ability at the mM level.

Relatively bulky organic molecules, such as sugars, can have little higher ·OH-scavenging abilities, while simple carbon chains, such as SDS, have lower ·OH-scavenging abilities compared to the other compounds tested. A spreading protein molecule such as gelatin can have higher ·OH-scavenging ability than a packed protein molecule such as albumin. Some compact molecules, such as DMSO and/or ethanol, can show relatively high ·OH-

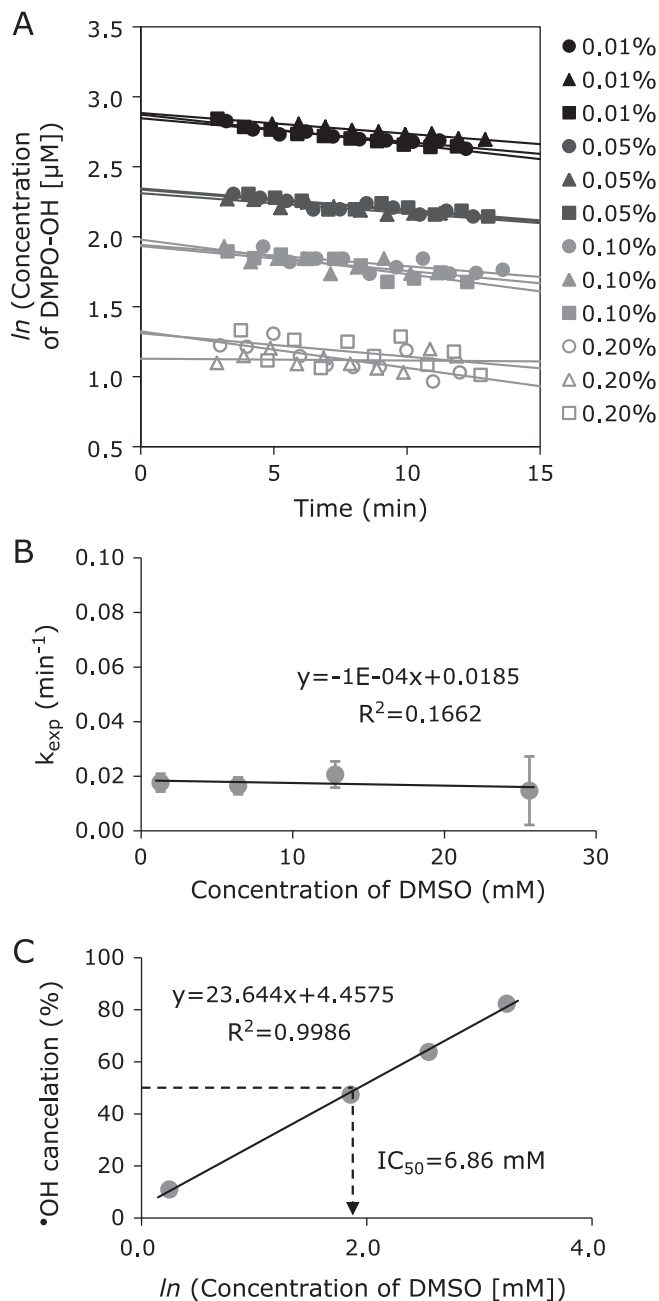


Fig. 2. Assessing antioxidative ability of DMSO. (A) Semi-logarithmic plot of time course of X-ray-induced DMPO-OH in the reaction mixture containing several DMSO concentrations. Decay rate of DMPO-OH, k_{exp} , and DMPO-OH amount at the end of X-ray irradiation, $C_{0\text{exp}}$, was estimated from the decay curve. (B) Estimating second order rate constant, $k_{2\text{nd}}$, of reducing DMPO-OH. Reduction rate of DMPO-OH, k_{exp} , at each concentration of DMSO was plotted versus the concentration, and then the second order rate constant, $k_{2\text{nd}}$, was obtained from the slope of the plot. (C) Estimating IC_{50} value of $\cdot\text{OH}$ -scavenging ability. Plotting the estimated $\cdot\text{OH}$ -scavenging ability with the logarithmic value of the DMSO concentration.

scavenging ability. Although highly reactive $\cdot\text{OH}$ may be able to react with any organic molecules basically, $\cdot\text{OH}$ even has likes and dislikes. Such organic compounds can abolish X-ray-induced $\cdot\text{OH}$ with mM concentration levels.

Next, $\cdot\text{OH}$ -scavenging abilities of some cell culture media were tested using the method proposed here. Cell culture media generally contain several organic compounds at relatively high

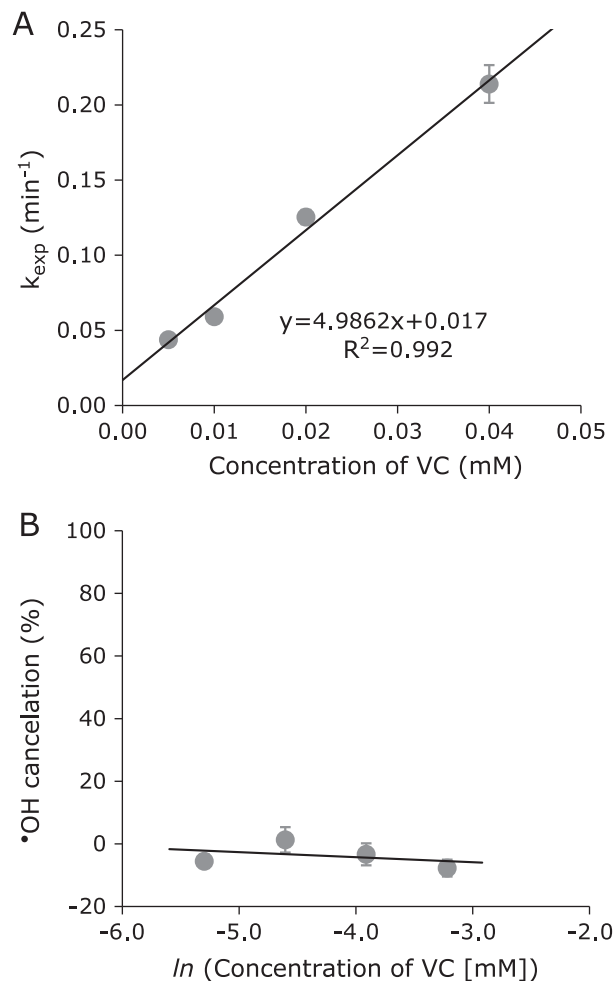


Fig. 3. Assessing antioxidative ability of VC. (A) Estimating second order rate constant, $k_{2\text{nd}}$, of reducing DMPO-OH by VC. (B) Estimating IC_{50} value of $\cdot\text{OH}$ -scavenging ability of VC, while no $\cdot\text{OH}$ -scavenging ability was obtained for VC at this concentration level.

concentrations. DMEM, RPMI-1640 medium, and Leibovitz's L-15 medium were tested in this paper. DMEM is a common medium for mammalian cell culture. RPMI-1640 medium is traditionally used for human lymphoid cells. Leibovitz's L-15 medium has been used for culturing osteoblasts and osteoclasts on fish scales.⁽¹⁸⁾ The results and concentration conditions are summarized in Table 2. DMEM containing a high concentration of glucose (4,500 mg/L) and Leibovitz's L-15 medium containing relatively high amino acid showed higher DMPO-OH-reducing ability, i.e. lower IC_{50} , than RPMI-1640 medium.

In addition, $\cdot\text{OH}$ -scavenging ability of rat plasma was tested using the method described above. The results are summarized in Table 3. When intact male rat plasma was used as the sample solution, EPR signal of DMPO-OH could not be obtained after X-ray irradiation. Reduction rate of DMPO-OH was significantly ($p < 0.001$) faster in male rat plasma to female rat plasma. In contrast, female rat plasma showed higher $\cdot\text{OH}$ -scavenging ability, i.e. lower IC_{50} value, than male rat plasma, while the difference was not statistically significant. The result in this experiment suggests that biological contents of both male and female rat plasma can abolish most X-ray-induced $\cdot\text{OH}$. In other words, most $\cdot\text{OH}$ generated in the living body can react easily with any biological molecules. This also means that $\cdot\text{OH}$ can become a trigger of oxidative stress if the $\cdot\text{OH}$ -scavenged product molecule is still reactive.

Table 2. $\cdot\text{OH}$ -scavenging and DMPO-OH-reducing ability of several cell culture mediums

Medium	Maker	IC ₅₀ (%)	k _{2nd} (min ⁻¹ % ⁻¹)
Dulbecco's Modified Eagle's Medium 10% Serum	SIGMA	43.26	0.0001
RPMI-1640 Medium 10% Serum	SIGMA	160.3	0.0001
Leibovitz's L-15 Medium [+] L-glutamine 2% penicillin-streptomycin mixture	GIBCO	48.96	NE

NE: The value could not be evaluated for the concentration range tested in this paper.

Table 3. $\cdot\text{OH}$ -scavenging and DMPO-OH-reducing ability of rat plasma

Gender	IC ₅₀ (%)	k _{2nd} (min ⁻¹ % ⁻¹)	n
Male	52.13 ± 10.8	0.0041 ± 0.0006	4
Female	43.59 ± 5.5	0.0016 ± 0.0005	4

Values are the means ± SD.

In this paper, the EPR spin-trapping technique was used for detection of $\cdot\text{OH}$. The EPR spin-trapping technique confirmed the detection of $\cdot\text{OH}$ due to the profile of the EPR spectrum; however, the method has some inapplicable targets. Since DMPO-OH is a nitroxyl radical, the method may be disrupted by hydrogen donors, such as NAD(P)H, and/or some thiol compounds, such as GSH.^(19,20) Applying other detection methods, such as luminescence probes, may make the method available for thiol compounds and other inapplicable cases.

Reaction rate constants of $\cdot\text{OH}$ and other molecules have been measured using the pulse radiolysis method.⁽²¹⁾ The reaction of hydroxyl radical is rapid in the order of 10^6 – 10^{10} M⁻¹s⁻¹. At biological concentrations (μM) of antioxidants, it is probably difficult to prevent such very fast reactions. Prevention of second, third, or later reactions may be important. To assess total reactions after X-ray irradiation the IC₅₀ values of DMPO-OH inhibition were examined in this paper.

Using the method proposed in this paper, antioxidative properties can be assessed by two factors, i.e. both $\cdot\text{OH}$ -scavenging and DMPO-OH-reducing. This method is good for estimating $\cdot\text{OH}$ -scavenging ability because X-ray-induced $\cdot\text{OH}$ generation in the

reaction mixture is not inhibited chemically. Correcting for the reduction of DMPO-OH during X-ray irradiation made the estimation of $\cdot\text{OH}$ -scavenging ability more accurate. The simultaneous estimation of $\cdot\text{OH}$ -scavenging and reducing ability of antioxidants can be an informative index for antioxidants.

The generation of $\cdot\text{OH}$ by X-ray irradiation of an aqueous reaction mixture cannot be inhibited chemically; therefore, the true $\cdot\text{OH}$ -scavenging ability of the subjected antioxidant can be assessed. In addition, the method simultaneously measured the reduction of a relatively stable radical, i.e. DMPO-OH, to estimate the reducing ability of the antioxidant. In this paper, $\cdot\text{OH}$ -scavenging and DMPO-OH-reducing abilities were estimated for various biologically common hydrophilic molecules, cell culture media, and rat plasma. Biological materials, such as sugars and proteins, could abolish $\cdot\text{OH}$ at the biological concentration. Cell culture media and rat plasma have relatively high $\cdot\text{OH}$ -scavenging ability.

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Conflict of Interest

No potential conflicts of interest were disclosed.

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