Effects of hydroxyl radicals generated from the depleted uranium-hydrogen peroxide systems

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Abstract

A complementary study of hydroxyl radical formation in the depleted uranium (DU)-hydrogen peroxide (H_2O_2) system and the effect of biosubstances on the system were examined using the spin-trapping method. Hydroxyl radical was formed in the uranyl ion (UO_2^{2+}), 5,5-dimethyl-1-pyrroline-N-oxide (DMPO), and hydrogen peroxide (H_2O_2) mixture solution. The pseudo first order rate constants of DMPO-OH formation were estimated to be 0.033 s^{-1} for UO_2^{2+}-H_2O_2-DMPO solution and 0.153 s^{-1} for UO^{2+}-H_2O_2-DMPO solution. Obtained results indicated that the hydroxyl radical formation in the UO_2^{2+}-H_2O_2 solution could be described as a stepwise reaction process including the reduction of UO_2^{2+} to UO_2^{+} by H_2O_2 and the Fenton-type reaction of UO_2^{+} with H_2O_2. Biosubstances, such as proteins, amino acids and saccharides, decreased the DMPO-OH formation, which was caused by the direct hydroxyl radical scavenging and the suppression of hydroxyl radical formation by coupling with uranyl ion.

Keywords: Depleted uranium toxicity, Hydrogen peroxide, Hydroxyl radical, ESR, Spin-trapping, Biosubstances

Introduction

Recently, the chemical toxicity of depleted uranium (DU) has become a focus of considerable interest due to the increase in its military use [1]. The main route of potentially hazardous exposure is inhalation. The uranyl ion derived from DU enters the systemic circulation, cleared to the blood, and then excreted through the kidneys [2,3]. Results obtained from long period studies using small animals concluded that a high dose of DU causes toxicity in the kidney tubules [4]. Uranium-238, the major radioisotope of DU, should decay with a 4.51×10^9 year of half life to produce α particles that will be absorbed around the irradiated surface. These considerations indicated that the radiation dose of DU is smaller than those of natural radon and its decay products. DU should, therefore, reveal a similar chemical toxicity to ordinal heavy metals, such as copper, cadmium and lead. Hamilton *et al.* studied the hydroxyl radical formation in DU-H_2O_2 systems and proposed a mechanism containing the reduction of U(VI) to U(IV) and a Fenton-type reaction [5]. Miller *et al.* showed DU-catalyzed oxidative DNA [6]. Both results support reactive oxygen species (ROS), such as hydroxyl radical, in the system as the cause of DU toxicity. In contrast, Yazzie *et al.* reported DNA strand cleavage by uranyl acetate in the presence of ascorbate [7], and discussed the possibility of the direct genotoxicity of DU, rather than through a Fenton-like reaction. These results indicate the diversity of the chemical toxicity of DU. In order to obtain further information on this chemical toxicity, the authors conducted a complementary study of hydroxyl radical formation in DU-H_2O_2 system, and also examined the effects of biosubstances on the system using the spin trapping method.

Experimental

Chemicals

5,5-Dimethyl-1-pyrroline N-oxide (DMPO) was obtained from DOJINDO Ltd.

(Kumamoto, Japan). DMPO was used without further purification [8]. Uranyl nitrate hexahydrate, $UO_2(NO_3)_2 \cdot 6H_2O$, was obtained from Merck & Co., Ltd. (Darmstadt, Fed. Rep. Germany). Other chemicals (guaranteed reagents) were obtained from Nacarai Tesque, Inc. (Tokyo, Japan) and Wako Pure Chemical Industries, Ltd. (Tokyo Japan). Uranous (UO^{2+}) solution was prepared as follows [9]: 1 g of granular zinc was added to 10 ml of uranyl nitrate solution (a mixture of 2.5 ml of 4×10^{-2} M uranyl nitrate and 7.5 ml of 5 M HCl) was let stand for 1 h, and then diluted to 25 ml with water. The formation of UO^{2+} was ascertained by UV-VIS absorption maximal peaks at 429, 494, 549, and 648 nm [10], using a UV-VIS spectrometer (V-550, JASCO, Tokyo, Japan).

Electron paramagnetic resonance measurements

Uranyl or uranous solution (10^{-3} M), DMPO (0.1 M), and H_2O_2 (10^{-2} M) were mixed. The solution pH was adjusted by using 0.1 - 1 M HNO_3 and NaOH solutions. The pH of the reaction mixture without pH adjustment was 4.0. After aspirating the mixture into a capillary tube, its ESR spectrum was immediately recorded using an X-band ESR spectrometer (JES TE-100, JEOL Ltd., Tokyo, Japan) controlled by an WIN-RAD ESR data analyzer (Radical Research Inc., Tokyo, Japan) under the following conditions: microwave power 5 mW; microwave frequency, 9.42 GHz; magnetic field, 335.3 mT; field sweep width, ± 5 mT; field modulation, 100 kHz; modulation width, 0.079 mT; sweep time, 1 min; response time, 0.1 s; and measurement interval, 2 min. As phosphate ion and HEPES (*N*-2-hydroxyethylpiperazine- *N'*-3-propanesufonic acid) heavily interfered the hydroxyl radical formation, these buffer reagents were not used for following experiments. A solution of H_2O_2 (10^{-2} M) and DMPO (10^{-2} M) in a capillary tube was irradiated with a glass fiber type UV irradiator (RUVF-203S, Radical Research Inc., Tokyo, Japan, Hg-Xe lamp, wavelength 365 nm, power 200 W) for 2 s. After irradiation, the ESR spectrum of the mixture solution was recorded. The amounts of DMPO-OH in the solution were almost the same as those in

the UO_2^{2+}-H_2O_2-DMPO solution. The DMPO-OH concentration was calibrated by using 2,2,6,6-tetramethyl-4-piperidinol-1- oxyl (Tempol), , as a standard, after double integration of the observed ESR signal.

Determination of uranium peroxide

Uranyl nitrate (10^{-3} M) and H_2O_2 (10^{-2} M) were mixed, and the pH was adjusted using 0.1 - 1.0 M HNO_3 and NaOH solutions. After the mixture was let stand for 1 h, the resulting precipitate was recovered by filteration using a membrane filter (pore size 0.2 μm). The amounts of uranium peroxide were estimated from the residual uranium in the filtrate determined by spectrophotometry using Arsenazo III [12].

Determination of dissolved oxygen concentration in the reaction mixture

The concentrations of oxygen in the reaction solutions, containing H_2O_2 and uranium ion, were monitored by electrochemical method, using a dissolved oxygen meter (DO-24P, DKK-TOA Corp. Tokyo, Japan).

Results

Kinetics of hydroxyl radical formation in DU-H_2O_2-DMPO solution

When uranyl nitrate (10^{-3} M), DMPO (0.1 M) and H_2O_2 (0.1 M) were mixed, an ESR signal of DMPO-OH, an adduct of hydroxyl radical (•OH), with four lines (intensity ratio, 1:2:2:1) appeared. Its g-value is 2.0066 and its hyperfine coupling constants, a_N = 1.49 mT, a_H = 1.49 mT). As shown in Fig. 1, ESR signal intensity of DMPO-OH in the UO_2^{2+}-H_2O_2-DMPO solution (pH 4) gradually increased with time, the time dependency of which was [1 - exp(-0.033t)]. One hour after the reaction was started, the amounts of

DMPO-OH in the solution reached about 10^{-5} M. The concentration of oxygen also gradually increased with time. Though the time dependency of DMPO-OH formation in the UO_2^{2+}-H_2O_2-DMPO solution (pH 1) was the same as that in the UO_2^{2+}-H_2O_2-DMPO solution (pH 4), the amounts of DMPO-OH reached about 2.5×10^{-5} M.

In contrast, the DMPO-OH signal intensity in the UO^{2+}-H_2O_2-DMPO solution (pH 1) increased rapidly within first 20 min, and then gradually decreased with time. The time dependency in its initial stage was formulated as [1 - exp(-0.153t)], the rate constant of which was five-times that in the UO_2^{2+}-H_2O_2-DMPO solution (pH 1 and 4). In addition, the maximum signal intensity of DMPO-OH increased to about 4×10^{-5} M, indicating that UO^{2+} ion forms DMPO-OH far more easily than UO_2^{2+} ion in the reaction with H_2O_2. The absorption maxima at 429, 494, 549, and 648 nm derived from UO^{2+} ion disappeared within few minutes after the addition of H_2O_2, and then a small peak at 415 nm derived from UO_2^{2+} ion appeared. These results confirmed that UO^{2+} ion was oxidized to UO_2^{+} by H_2O_2 through a Fenton-type reaction. As the absorption peaks for UO_2^{+} ion were also not observed, the ion is therefore unstable and oxidizes to UO_2^{2+} immediately.

Profile of the DMPO-OH formation in UO_2^{2+}-H_2O_2-DMPO solution

The pH dependency of the DMPO-OH signal intensity in the UO_2^{2+}-H_2O_2-DMPO solution was plotted in Fig. 2 in comparison with that in the UV-irradiated-H_2O_2-DMPO solution. The ESR signal intensity of DMPO-OH in the UO_2^{2+}-H_2O_2-DMPO solution showed a maximum (7.9×10^{-5} M) at pH 1.5, and a rapid decrease above and below this pH. Hamilton *et al.* reported that hydroxyl radical formation was increased in the narrow pH range around 0.6 (pH of metal solution) [5]. As the final pH of reaction solution was adopted in this experiment, the pH range was different from that of Hamilton *et al.* In the pH range from 4 to 8, only small amounts of DMPO-OH were seen (10^{-5} M). In contrast, the DMPO-OH signal

intensity in the UV-irradiated-H_2O_2 system increased with pH, reached a plateau above pH 2, and then gradually decreased. These results indicated that the decrease of the DMPO-OH signal intensity in the UO_2^{2+}-H_2O_2-DMPO solution below pH 1.5 is caused by instability of the DMPO-OH due to irreversible protonation of the radical center. The reaction solution containing 10^{-3} M of UO_2^{2+} and 0.1 M of H_2O_2, resulted the precipitation of uranium peroxide, $UO_4 \cdot 2H_2O$ [10]. The pH dependence of the precipitation ratio is also depicted in Fig. 2. At pH 3.6 and 2.6, the precipitation ratios were found to be 98 and 95 %, respectively, close to that at pH 4. The ratio showed dramatic decrease to 6 % at pH 1.6. This observation relates to the significantly low DMPO-OH signal intensity in the UO_2^{2+}-H_2O_2-DMPO solution at pH 4.

The concentration of DMPO-OH radical observed after 60 min in the UO_2^{2+}-H_2O_2-DMPO solution(10^{-3} M of UO_2^{2+}) was plotted against the concentration of H_2O_2 (Fig. 3). The concentration of the radical revealed a steep increase and became constant above 2.5×10^{-4} M H_2O_2. This constant value was almost equal to that observed in the UO_2^{2+}-H_2O_2-DMPO solution (10^{-3} M UO_2^{2+} and 0.1 M H_2O_2). These observations indicate that excess H_2O_2 above 2.5×10^{-4} M depresses hydroxyl radical formation by the precipitation of $UO_4 \cdot 2H_2O$, as mentioned above. The solubility product of $UO_4 \cdot 2H_2O$, $[UO_2^{2+}][H_2O_2]$, can be estimated as 2.5×10^{-7} M^2 at pH 4.

Effect of biosubstances on hydroxyl radical formation in UO_2^{2+}-H_2O_2 system

The degradation of hydroxyl radical formation was examined using ID_{50} (mg/ml), the concentrations of biosubstances when the amounts of DMPO-OH is the half of those without biosubstances (Table 1). The order of ID_{50} for proteins was ovalbumin << milk casein << gelatin in UO_2^{2+}-H_2O_2-DMPO solution and ovalbumin << gelatin < milk casein in UV irradiated-H_2O_2-DMPO solution. ID_{50} of amino acids were almost same values both in UO_2^{2+}-H_2O_2-DMPO and UV irradiated-H_2O_2-DMPO solutions. ID_{50} of saccharides in

UO_2^{2+}-H_2O_2-DMPO solution were larger than those in UV irradiated-H_2O_2-DMPO solution. ID_{50} of microbial cells, such as *Arthrobacter nicotianae* (IAM 12342) and *Zooglea nicotianae* (IAM 12136), were almost similar values as those of milk casein. As a whole, ovalbumin indicated the highest value of ID_{50}.

Discussion

Possible mechanism of hydroxyl radical formation in DU-H_2O_2 system was considered from the present and Hamilton's results. Hamilton *et al.* proposed a mechanism containing the reduction of U(VI) to U(IV) and a Fenton-type reaction [5]. Uranyl ion, UO_2^{2+}, being the highest oxidation state of uranium, could not react with H_2O_2 to produce hydroxyl radical. Thus, the reduction of UO_2^{2+} to UO_2^{+} and/or UO^{2+} should be needed. The production of molecular oxygen in the solution suggested the reduction of UO_2^{2+} to UO_2^{+}. In the solution, H_2O_2 should function only as an agent for the reduction of UO_2^{2+} to UO_2^{+}. In the UO^{2+}-H_2O_2-DMPO solution, hydroxyl radical was also formed in amounts about twice those in the UO_2^{2+}-H_2O_2-DMPO solution (pH 1). It is, therefore, possible to propose the following reaction scheme for DMPO-OH formation in the UO_2^{2+}-H_2O_2-DMPO solution:

(a) $2UO_2^{2+} + H_2O_2 \rightarrow 2UO_2^{+} + 2H^{+} + O_2\uparrow$ (reduction) (1)

(b) $2UO_2^{+} + H_2O \rightarrow UO^{2+} + UO_2^{2+} + 2OH^{-}$ (disproportionation) (2)

(c) $UO_2^{+} + H_2O_2 \rightarrow UO_2^{2+} + \bullet OH + OH^{-}$ (Fenton-type reaction) (3)

(d) $UO^{2+} + H_2O_2 \rightarrow UO_2^{+} + \bullet OH + H^{+}$ (Fenton-type reaction) (4)

(d) $DMPO + \bullet OH \rightarrow DMPO\text{-}OH$ (spin-trapping reaction) (5)

(e) $UO_2^{2+} + H_2O_2 + 2H_2O \rightarrow UO_4{\cdot}2H_2O \downarrow + 2H^{+}$ (precipitation) (6)

The hydroxyl radical-trapping reaction of DMPO is very rapid (Equation 6; rate constant $k_0 = 2.1 \times 10^9\ M^{-1}s^{-1}$) [13], and the trapping ratio of hydroxyl radical is almost 100 % [14], which supports the concentration of DMPO-OH directly corresponding to the hydroxyl

radical formation. The disproportionation, Equation 3, was so slow at pH 2 - 4 that Equation 5 should be negligible in the UO_2^{2+}-H_2O_2-DMPO solution [10]. The optical absorption spectra of the UO_2^{2+}-H_2O_2 solution indicated the conservation of the peak at 415 nm for UO_2^{2+} ion throughout the reaction. In the UO^{2+}-H_2O_2-DMPO solution, two Fenton-type reaction steps, Equations 3 and 4, should occur, and the double amounts of DMPO-OH formed in the UO_2^{2+}-H_2O_2-DMPO solution (pH 1) were resulted. The initial reaction stage of the DMPO-OH radical formation proceeded as pseudo first order reaction under excess amounts of H_2O_2 in the solution. The rate constant of the DMPO-OH formation in the UO_2^{2+}-H_2O_2-DMPO solution at pH 4 is estimated to be 0.033 s^{-1}. As the rate constant of the Fenton-type reaction, Equation 3, should be 0.153 s^{-1} or faster, the rate constant of the reduction of UO_2^{2+} to UO_2^{+}, Equation 2, become the rate determining step and its rate constant should be 0.033 s^{-1}.

Summarizing these considerations, •OH should be formed through the reduction of UO_2^{2+} to UO_2^{+} (Equation 1) and the following Fenton-type reaction (Equation 3). The whole reaction in the solution was summarized as follows.

$$UO_2^{2+} + (3/2)H_2O_2 \rightarrow UO_2^{2+} + \bullet OH + H_2O + (1/2)O_2\uparrow \quad (7)$$

The proposed reaction scheme indicates that the red-ox reaction of UO_2^{2+} ion should be repeated in the presence of H_2O_2. Uranyl ion, therefore, produces hydroxyl radical continuously for several hours in the presence of 10^{-6} - 0.1 M of H_2O_2.

As the concentration of DMPO-OH directly corresponding to the hydroxyl radical formation, ID_{50} indicates the abilities of the depression of the radical formation. As shown in Table 1, ovalbumin has the highest ability to depress the radical formation. Ovalbumin, milk casein and microbial cells depressed the radical formation in the UO_2^{2+}-H_2O_2-DMPO solution more heavily than in UV-irradiated-H_2O_2-DMPO. These results suggested that the decrease of the radical formation by biosubstances will be caused by two processes, (1) direct scavenging of hydroxyl radical competing with the spin-trapping reaction (Equation 5), (2) depression of

the radical formation by coupling with UO_2^{2+} competing with the reduction of UO_2^{2+} to UO_2^{+} (Equation 1). In ovalbumin, milk casein and microbial cells, the latter interaction should be important, which consists with previous results on the UO_2^{2+} coupling with protein-tannin compounds [15]. Both processes are possible to raise the chemical toxicity of depleted uranium.

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Table 1. Effect of biosubstances on the formation of hydroxyl radical generated in UO_2^{2+}-H_2O_2 and UV irradiated-H_2O_2 systems.

Biosubstances	ID_{50}			
	UO_2^{2+}-H_2O_2 [(a)]		UV irradiated-H_2O_2 [(b)]	
	M	g/liter	M	g/liter
(Proteins)				
Ovalubumin		0.059+/-0.004		0.75+/-0.05
Milk casein		0.35+/-0.06		2.9+/-0.5
Gelatin		3.6+/-0.4		1.9+/-0.2
(Microbial cells)				
Arthrobacter nicotianae (IAM 12342)		0.28+/-0.03		2.5+/-0.3
Zooglea ramigera (IAM 12136)		0.14+/-0.01		1.5+/-0.1
(Amino acids)				
Glycine	0.141+/-0.006	9.2+/-0.4	0.79+/-0.04	59+/-3
Glycylglycine	0.031+/-0.005	4.1+/-0.7	0.031+/-0.002	4.1+/-0.3
Glycylglysylglycine	0.018+/-0.003	3.4+/-0.6	0.021+/-0.001	4.0+/-0.2
α-Alanine	0.076+/-0.005	6.8+/-0.5	0.064+/-0.003	5.7+/-0.3
Alanylalanine	0.032+/-0.005	5.1+/-0.8	0.0098+/-0.0003	1.6+/-0.05
Alanylalanylalanine	0.023+/-0.002	5.3+/-0.5	0.0074+/-0.0004	1.7+/-0.1
L-Histidine	0.062+/-0.006	9.6+/-0.9	0.0019+/-0.0002	0.29+/-0.03
L-Methionine	0.036+/-0.005	5.4+/-0.7	0.0012+/-0.0001	0.19+/-0.02
(Saccharides)				
D-Mannitol	0.12+/-0.02	22+/-4	0.0030+/-0.0003	0.63+/-0.06
D-Glucose	0.18+/-0.04	33+/-7	0.0035+/-0.0004	1.4+/-0.2
D-Glucosamine	0.11+/-0.01	20+/-2	0.0076+/-0.0006	0.68+/-0.05
D-Ribose	0.25+/-0.04	37+/-6	0.0045+/-0.0006	0.75+/-0.10
2-Deoxy-D-ribose	0.21+/-0.02	28+/-3	0.0056+/-0.0007	0.55+/-0.07

(a) Biosubstances (0 - 1 M or 0 - 100 g/ liter), UO_2^{2+} (10^{-3} M), DMPO (0.1 M), and H_2O_2 (10^{-2} M) were mixed.

(b) Biosubstances (0 - 1 M or 0 - 100 g/ liter), DMPO (0.1 M), and H_2O_2 (10^{-2} M) were mixed, and then was irradiated UV (365 nm).

ID_{50} indicated the concentrations of biosubstances when the amounts of DMPO-OH is the half of those without biosubstances. For biosubstances with molecular formula, both units, M (mol/liter) and g/liter, were represented.

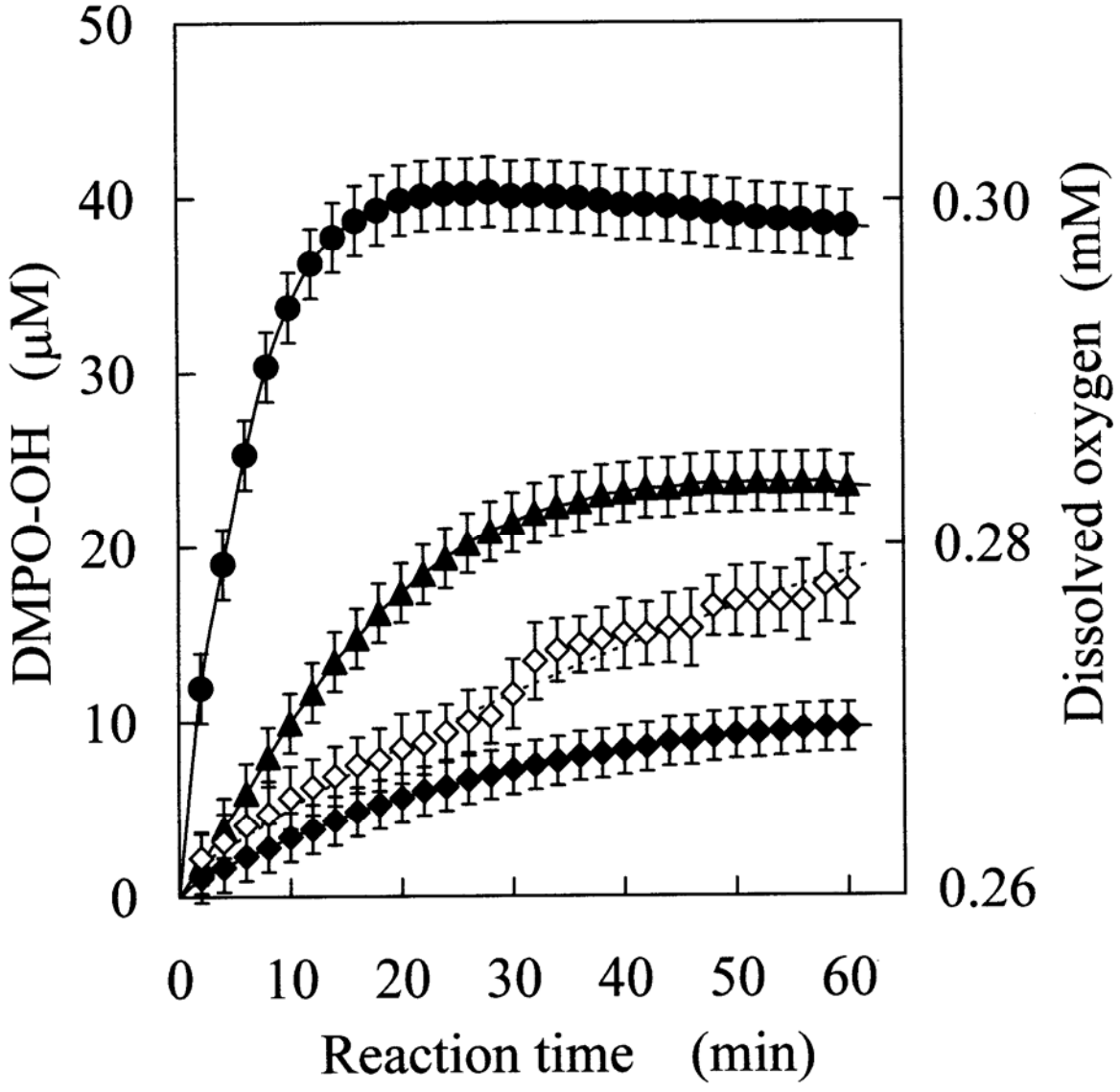

Fig. 1. Time course of DMPO-OH formation in metal ion-H_2O_2-DMPO solutions. Uranium ion (10^{-3} M), H_2O_2 (0.1 M), and DMPO (0.1 M) were mixed. Closed circle indicates the UO^{2+}-H_2O_2-DMPO solution (pH 1), closed triangle, the UO_2^{2+}-H_2O_2-DMPO solution (pH 1), closed lozenge, and the UO_2^{2+}-H_2O_2-DMPO solution (pH 4), and open lozenge, the dissolved oxygen in the UO_2^{2+}-H_2O_2 solution (pH 4).

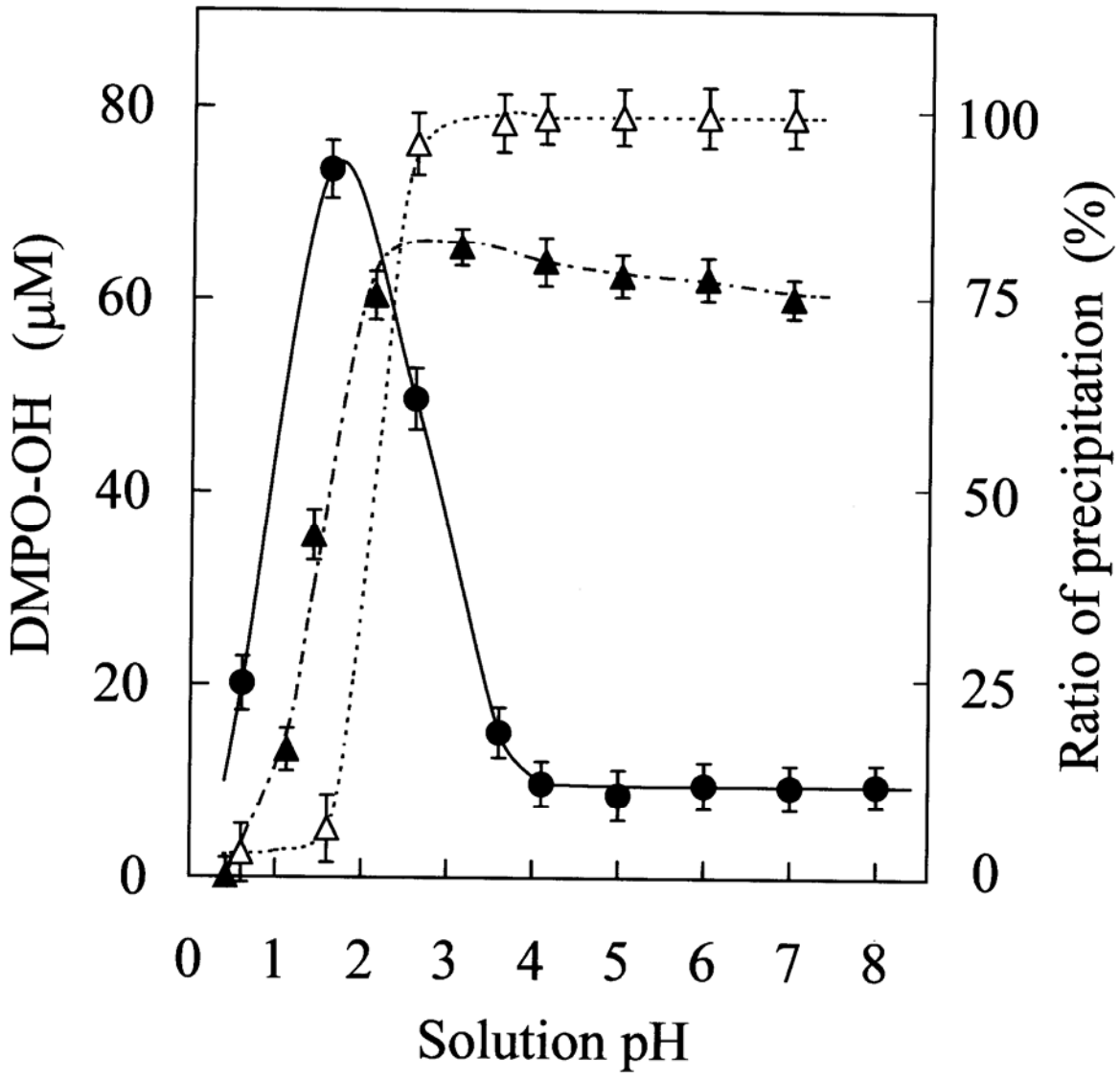

Fig. 2. Effect of solution pH on the formation of DMPO-OH in the UO_2^{2+}-H_2O_2-DMPO solution. Uranyl ion (10^{-3} M), H_2O_2 (0.1 M), and DMPO (0.1 M) were mixed, the pH of which was adjusted with HNO_3. Closed circle indicated the DMPO-OH formed in the solution. Closed triangle indicates the DMPO-OH formed in the UV irradiated-H_2O_2-solution (10^{-2} M H_2O_2 and 10^{-2} M DMPO), open triangle, the ratio of the precipitation of uranium peroxide.

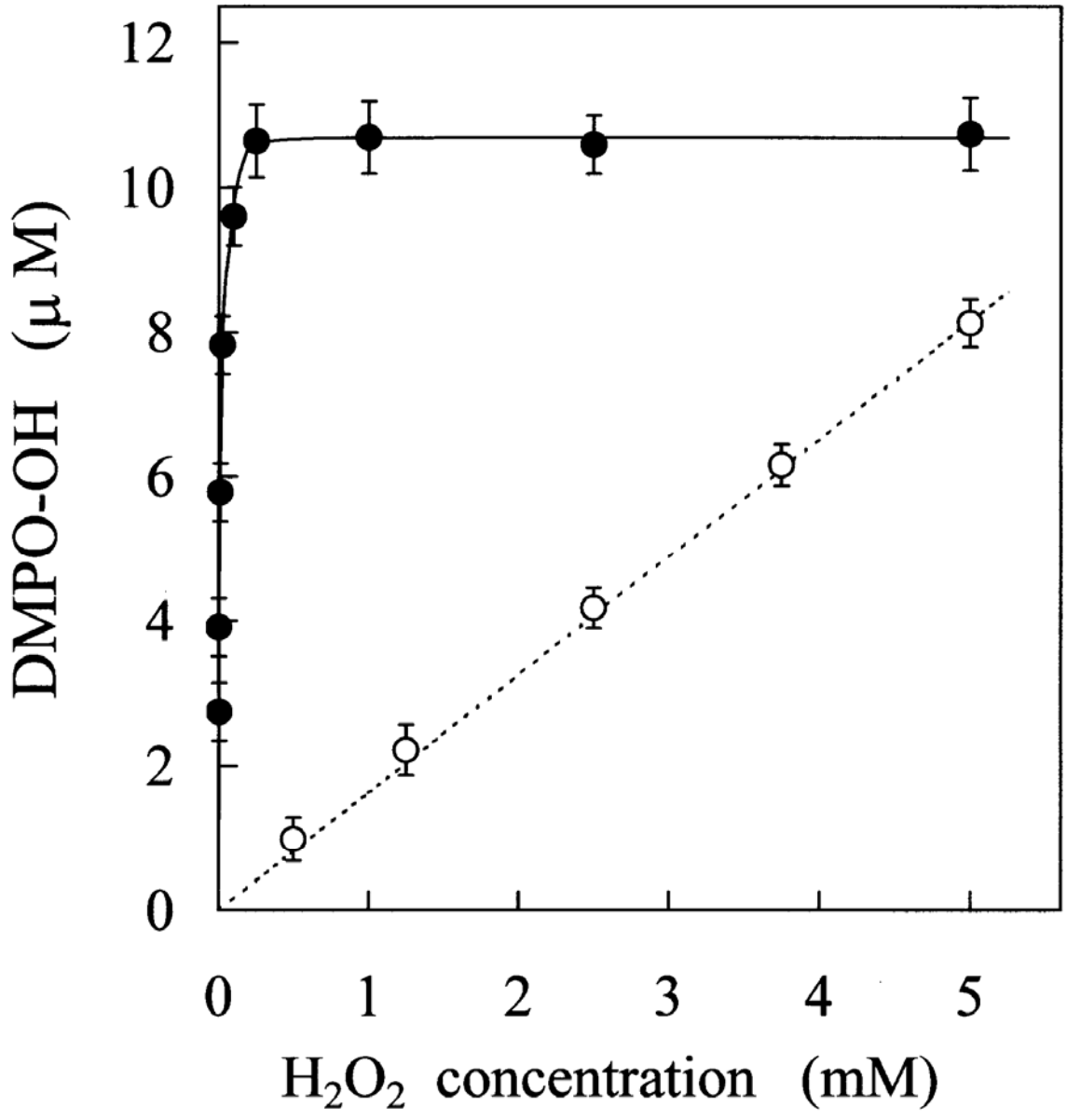

Fig. 3. Effect of the concentration of hydrogen peroxide on the DMPO-OH formation in the UO_2^{2+}-H_2O_2-DMPO and UV-irradiated H_2O_2-DMPO solutions. Uranyl ion (10^{-3} M), H_2O_2 (0.25 - 5.0 × 10^{-3} M), and DMPO (0.1 M) were mixed at pH 4 (closed circle). The mixture of H_2O_2 (10^{-5} - 5 × 10^{-3} M) and DMPO (10^{-2} M) were irradiated for 2 s (open circle).