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Spin-Trapping of Selenium-Containing Inorganic Radicals in Aqueous Solutions

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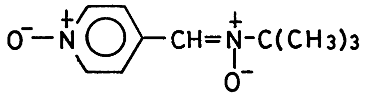
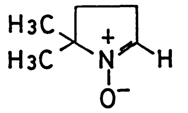
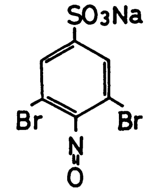
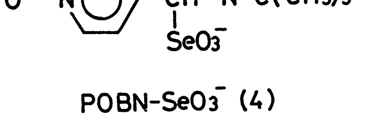
Synopsis. The selenite radical anion, $SeO_3^{\cdot -}$, can be detected by ESR spectroscopy using water-soluble spin-traps, *N*-(4-pyridylmethylene)-*t*-butylamine *N,N'*-dioxide (POBN) and 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS), but selenate radical anion, $SeO_4^{\cdot -}$, cannot be trapped by POBN or DBNBS.

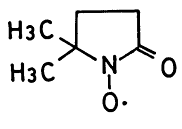
In continuation of our works[1–4] on the spin-trapping of short-lived inorganic radicals, we have investigated the spin-trapping of selenium-containing inorganic radicals. Selenium is being increasingly recognized as biochemical importance.[5] It is now recognized to be an essential nutrient and is present, for example, in glutathione peroxidase, an enzyme important in the metabolism of hydroperoxides. It also has come out as a significant anticarcinogen. However, the chemistry *per se* is still not understood.[6] Selenium can exist as selenide (Se^{2-}), elemental selenium (Se^0), selenite (SeO_3^{2-}), and selenate (SeO_4^{2-}). Each oxidation state exhibits different chemical behavior. Metal selenides, Se-sulfides, and elemental Se are insoluble, and therefore biologically unavailable. For the pH and redox conditions of aquatic environments, SeO_3^{2-} and SeO_4^{2-} should be dominant forms of Se. Consequently, it is imperative to know the chemical reactivities of these ions. If these ions are oxidized by one electron oxidation, these may produce radical intermediates. However, at present there have been no reports concerning the spin-trapping of radical intermediates formed during the oxidation of the selenium-containing inorganic compounds, the lifetime of which is too short to be detected by conventional ESR measurements. It has been reported that spin-trapping is a technique to detect short-lived free radicals in solutions. That is, this technique involves the ability of spin-trap to react with short-lived free radicals to yield a spin adduct having a longer lifetime, the ESR spectrum of which gives information specially identifying the original radical species.

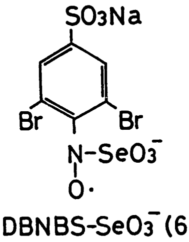

POBN(1)


DMPO(2)


DBNBS(3)


POBN-SeO_3^- (4)


DMPOX(5)


DBNBS-SeO_3^-(6)

It is known that Ce(IV) is a strong oxidizing agent in acidic aqueous solutions. Then, using this ion as an oxidizing agent, we have examined the spin-trapping of selenium-containing inorganic radicals. We now first report herein selenium-containing inorganic radicals trapped by water-soluble spin-trap.

Experimental

Materials. Water-soluble spin-traps, *N*-(4-pyridylmethylene)-*t*-butylamine *N,N'*-dioxide (POBN, **1**) and 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO, **2**), were purchased from Sigma Chemical Co. and used without further purification, because no ESR signal was observed when these spin-traps were dissolved in an acidic aqueous solution. Other water-soluble spin-trap, sodium 3,5-dibromo-4-nitrosobenzenesulfonate (DBNBS, **3**) was synthesized as described previously.[7] The selenium compounds, sodium selenite (Na_2SeO_3) and sodium selenate (Na_2SeO_4) were obtained from Wako Pure Chemical Ind., Ltd. and used as received. $Ce(NO_3)_4 \cdot 2NH_4NO_3$ was purchased from Wako Pure Chemical Ind., Ltd. and used as a source of Ce(IV). Other reagents were commercially available. All reaction solutions were prepared from deionized and triply distilled water and their pHs were adjusted to be 2.0 by sulfuric acid.

Measurements. ESR measurements were carried out on a JEOL JES-RE-1X ESR spectrometer (X-band) with 100 kHz field modulation. ESR spectra were recorded at room temperature in a JEOL flat quartz cell. ESR parameters were calibrated by comparison with a standard Mn^{2+}/MgO marker and 1,1-diphenyl-2-picrylhydrazyl (DPPH, g=2.0036). Further, magnetic field was calibrated with NMR field meter (JEOL ES-FC5).

Results and Discussion

Spin-Trapping with POBN. When Ce(IV) solution (1 mmol dm^{-3}) was mixed with the aqueous solution of selenite ion (SeO_3^{2-}) (10 mmol dm^{-3}) containing POBN (10 mmol dm^{-3}), ESR spectrum as shown in Fig. 1 was

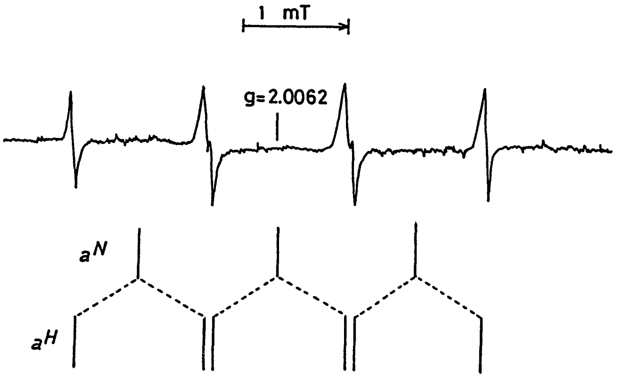


Fig. 1. ESR spectrum observed during the oxidation of SeO_3^{2-} with Ce(IV) in the presence of POBN. Conditions: Ce(IV), 1 mmol dm^{-3}; SeO_3^{2-}, 10 mmol dm^{-3}; POBN, 10 mmol dm^{-3}.

observed. The ESR spectrum could be analyzed as shown by the stick diagram in Fig. 1 and ESR parameters obtained were as follows: $a^N(1)=1.49$ mT, $a^H(1)=1.39$ mT, $g=2.0062$. When either Ce(IV) or SeO_3^{2-} ion was omitted from the solution, no ESR spectrum was observed. Further, Ce(IV) is used as an one-electron oxidant. The potential of Ce(IV)/Ce(III) couple in 1 M H_2SO_4 (1 M=1 mol dm^{-3}) solution is as follows:[8]

$$\mathrm{Ce(IV)} + \mathrm{e} = \mathrm{Ce(III)} \qquad E^0 = +1.44\ \mathrm{V} \tag{1}$$

Therefore, the observed radical species is assigned to SeO_3^- adduct of POBN (**4**). The formation of (**4**) can be expressed as follows:

$$\mathrm{Ce(IV)} + \mathrm{SeO_3^{2-}} \longrightarrow \mathrm{Ce(III)} + \mathrm{SeO_3^{\overline{\cdot}}} \tag{2}$$

$$\mathrm{SeO_3^-} + \mathrm{POBN} \longrightarrow (\mathbf{4}) \tag{3}$$

In this case, hyperfine splitting from ^{75}Se ($I=1/2$, natural abundance 7.50%) was not observed.

On the other hand, no ESR spectrum was observed from selenate ion (SeO_4^{2-}) by oxidation with Ce(IV). The yellow color of aqueous Ce(IV) solutions, however, changed to colorless immediately after additon of selenate solutions, suggesting that Ce(IV) was reduced to Ce(III). Therefore, this result may be considered that POBN cannot form the stable adduct with selenate anion radical, $SeO_4^{\overline{\cdot}}$.

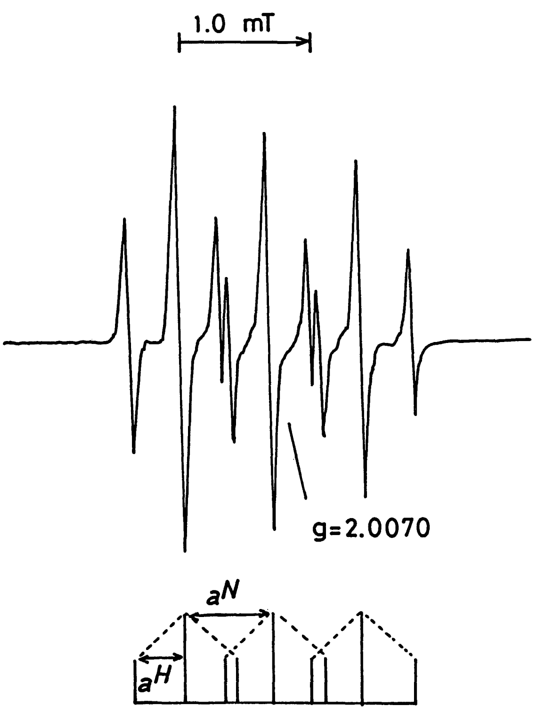


Fig. 2. ESR spectrum obtained during the oxidation of SeO_3^{2-} by Ce(IV) in the presence of DMPO. Conditions: Ce (IV), 1 mmol dm^{-3}; SeO_3^{2-}, 10 mmol dm^{-3}; DMPO, 10 mmol dm^{-3}.

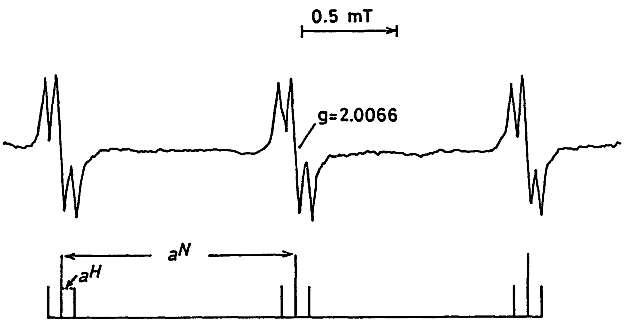


Fig. 3. ESR spectrum observed from the oxidation of SeO_3^{2-} by Ce(IV) in the presence of DBNBS. Conditions: Ce(IV), 1 mmol dm^{-3}; SeO_3^{2-}, 10 mmol dm^{-3}; DBNBS, 10 mmol dm^{-3}.

Spin-Trapping-with DMPO. ESR spectrum was observed as shown in Fig. 2, when Ce(IV) solution (1 mmol dm^{-3}) was mixed with aqueous solutions of selenite ion (10 mmol dm^{-3}) containing DMPO (10 mmol dm^{-3}). The ESR spectrum could be analyzed as shown by the stick diagram in Fig. 2 and ESR parameters obtained were as follows: $a^N(1)=0.74$ mT, $a^H(2)=0.41$ mT, and $g=2.0070$. ESR spectrum shown in Fig. 2 could not be observed, when either Ce(IV) or selenite ion was omitted from the reaction solution. This ESR spectrum was identical with that of 5,5-dimethyl-2-oxol-1-pyrrolidinyloxyl (DMPOX, **5**)[9] which was generated by the oxidation of DMPO with α-cumyl hydroperoxide–hematin system. Thus, the radical species giving ESR spectrum shown in Fig. 2 can be assigned to DMPOX. The same ESR spectrum as shown in Fig. 2 was observed when the selenate ion was oxidized by Ce(IV) in the presence of DMPO. Rosen et al. suggsted that DMPOX arises by spin-trapping α-cumyldioxyl radicals followed by base-catalyzed rearrangement.[10] Thus, according to their suggestion, DMPOX may arise by spin-trapping selenite anion radical or selenate anion radical followed by base-catalyzed rearrangement. However, since it is known that the formation mechanism of DMPOX is very complicated,[11] at present the exact reaction scheme is not explained.

Spin-Trapping with DBNBS. When Ce(IV) solution (1 mmol dm^{-3}) was mixed with the aqueous solution of selenite ion (SeO_3^{2-}) (10 mmol dm^{-3}) including DBNBS (10 mmol dm^{-3}), ESR spectrum was observed as shown in Fig. 3. The ESR spectrum could be analyzed as shown by the stick diagram in Fig. 3 and ESR parameters obtained were as follows: $a^N(1)=1.24$ mT, $a^H(2)=0.07$ mT, $g=2.0066$. This ESR spectrum could not be observed, when either Ce(IV) or selenite ion was ommited from the reaction solution. Further, the hyperfine splitting constants are similar to the spin adducts of DBNBS with sulfur-containing inorganic radicals such as $SO_3^{\bar{\cdot}}$,1) $S_2O_3^{\bar{\cdot}}$, and $S^{\bar{\cdot}}$.4) These results suggest that the radical species observed here are assignable to the spin adduct of DBNBS with $SeO_3^{\bar{\cdot}}$ radical (**6**). In this case, hyperfine splitting due to ^{75}Se was not observed.

On the other hand, no ESR spectrum was obtained from selenate ion (SeO_4^{2-}) by oxidation with Ce(IV). Since Ce(IV) is reduced by SeO_4^{2-} to Ce(III) as described previously, $SeO_4^{\bar{\cdot}}$ radical may be formed. Thus, DBNBS may not give a stable spin adduct with $SeO_4^{\bar{\cdot}}$.

In our experiments, $SeO_4^{\bar{\cdot}}$ radical could not be trapped by water-soluble spin-traps used here. In this case, it is very difficult to distinguish whether $SeO_4^{\bar{\cdot}}$ radical was not generated from the oxidation of SeO_4^{2-} ion with Ce^{4+} or the spin adducts of $SeO_4^{\bar{\cdot}}$ radical was too unstable to be detected by ESR. To elucidate this problem it will be needed to use the some radical scavenger for $SeO_4^{\bar{\cdot}}$ radical, although we can not find out such radical scavenger at present time.

In conclusion, selenite anion radical, $SeO_3^{\bar{\cdot}}$, was first observed by ESR using water-soluble spin-traps, POBN and DBNBS. But, $SeO_4^{\bar{\cdot}}$ cannot be trapped by both spin-traps. Using DMPO as a spin-trap for both $SeO_3^{\bar{\cdot}}$ and $SeO_4^{\bar{\cdot}}$ radicals, oxidation product, DMPOX was formed instead of spin adduct of DMPO.

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