ESR EVIDENCE FOR THE FORMATION OF HYDROXYL RADICALS DURING THE REACTION OF VANADYL IONS WITH HYDROGEN PEROXIDE

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Hydroxyl radicals from the reaction of vanadyl ions (VO^{2+}) with hydrogen peroxide (H_2O_2) in an acidic solution can be detected by an electron spin resonance (ESR) spectroscopy using water-soluble spin-traps, α-(4-pyridyl-1-oxide)-N-tert-butylnitrone (POBN) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO). Further, the fact that some hydroxyl radical scavengers suppressed or reduced the production of POBN-OH adducts affirms the formation of hydroxyl radicals at the initial step of the reaction between VO^{2+} and H_2O_2.

KEYWORDS hydroxyl radical; vanadyl ion; hydrogen peroxide; spin-trap; ESR; α-(4-pyridyl-1-oxide)-N-tert-butylnitrone; POBN; 5,5-dimethyl-1-pyrroline N-oxide; DMPO; radical scavenger

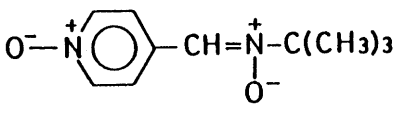
Recently, the biological effects of vanadium compounds have been extensively reviewed,[1,2] but the exact mechanism of vanadium toxicity is unknown. The report of a vanadate-dependent NADH oxidase present in cardiac cell membranes[3] created speculation that vanadium toxicity may involve changes in the cellular redox states. Further, several studies have shown that NAD(P)H oxidase activity occurs in a variety of membranes,[4-6] but this oxidation can also occur non-enzymatically.[6,7] In non-membrane oxidation, introduction of superoxide (O_2^-) generated from enzymatic, photochemical and chemical sources can stimulate NADH oxidation by vanadate via a free radical chain mechanism.[8-10] These studies indicate that the ability of vanadate to oxidize NAD(P)H in systems containing biomembranes may reflect superoxide production by these membranes.[11]

More recently, Piette et al. reported that vanadyl ion (VO^{2+}) is the active form of vanadium in stimulating NADH oxidation, and that in the presence of superoxide, vanadyl ions can react in a Fenton-type mechanism to produce hydroxyl radicals (·OH).[12] They suggested that the formation of hydroxyl radicals is a significant factor in any condition involving vanadyl ions that increases NADH oxidation. The ability of vanadyl ions to generate hydroxyl radicals may prove to be a major factor in the toxicity of vanadium. In the living body, hydrogen peroxide (H_2O_2) may be formed by dismutation of superoxide. Thus, it is very important to know the reaction of vanadyl ion with H_2O_2 in connection with the toxicity of vanadium.

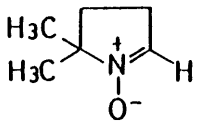
Previously, we reported that the radical complex, VO_2^+-HO_2·, generated during the reaction of VO^{2+} with H_2O_2 in an acidic solution, was observed by using a continuous flow-ESR technique and assumed that in this reaction process hydroxyl radicals were formed at an initial step in the reaction.

In this communication, we report that hydroxyl radicals can be detected by an ESR spectrometer using water-soluble spin-traps, α-(4-pyridyl-1-oxide)-N-tert-butylnitrone (POBN, 1) and 5,5-dimethyl-1-pyrroline N-oxide (DMPO, 2) during the reaction of VO^{2+} ions with H_2O_2.[13,14]

Vanadyl sulfate ($VOSO_4$) purchased from Aldrich Chemical Co. was used as a source of VO^{2+} ions. Spin-traps, POBN and DMPO, were obtained from Sigma Chemical Co. POBN was used without further purification. DMPO was purified before use by the method described in the literature.[15] H_2O_2 (35%) was purchased from Wako Pure Chemical Co. and determined by titration with $KMnO_4$. Other reagents were



POBN(1)



DMPO(2)

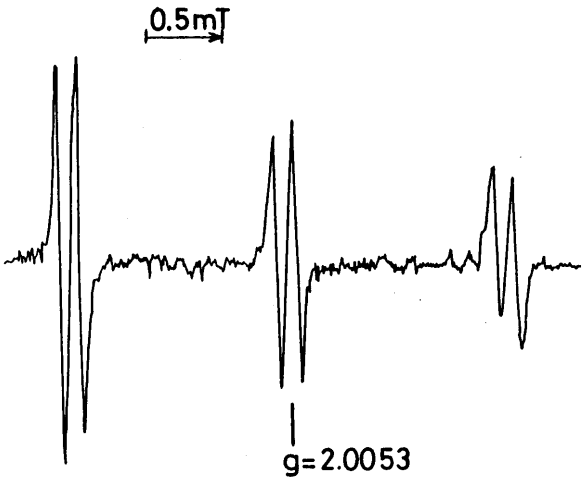


Fig. 1. ESR Spectrum Observed during the Reaction of VO^{2+} Ions with H_2O_2 in the Presence of POBN
Concentrations: VO^{2+}, 1 mmol; H_2O_2, 10 mmol; POBN, 5 mmol.

commercially available. The reaction solutions were prepared from deionized and triply distilled water and adjusted to pH 2.0 with ammonium hydroxide and sulfuric acid.

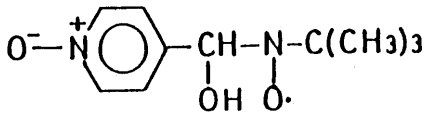
ESR measurements were carried out on a JEOL-PE-1X ESR spectrometer (X-band) with 100 kHz field modulation. ESR spectra were recorded at room temperature with a JEOL flat quartz cell. ESR parameters were calibrated by comparison with a standard Mn^{2+}/MgO marker and 1,1-diphenyl-2-picrylhydrazyl (DPPH,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

An acidic solution of VO^{2+} ions (1 mmol) showed an ESR spectrum consisting of eight resonance lines ($a^{V(IV)}(1)$ = 11.61 mT, g = 1.978) due to V(IV) ions (I = 7/2).[16] This ESR signal completely disappeared when it was mixed with hydrogen peroxide (10 mmol), but no new ESR signal appeared, because the intermediate involved in the reaction solutions was a very short-lived VO_2^+-$HO_2\cdot$ complex radical (less than 100 msec) which can be detected only by a continuous flow method.[14] However, when an aqueous solution of H_2O_2 (10 mmol) containing POBN (5 mmol) was mixed with an aqueous solution of VO^{2+} ions (1 mmol), a doublet of the triplet ESR signal was observed as shown in Fig. 1. The mixture of POBN and H_2O_2 did not show any ESR spectrum. Further, this ESR spectrum is the same as that of the POBN-OH adduct (3) which was formed by Fenton reaction (Fe^{2+}-H_2O_2) in the presence of POBN.[17] Therefore, the ESR spectrum shown in Fig. 1 is assignable to the POBN-OH adduct (3) ($a^N(1)$ = 1.52 mT, $a^H(1)$ = 0.24 mT and g = 2.0053). Since in an acidic solution less than pH 2, vanadium(V) ion exists mainly as dioxonanadium ions (VO_2^+),[18] the POBN-OH adduct (3) may be formed as shown in equations (1-2).

$$VO^{2+} + H_2O_2 \longrightarrow VO_2^+ + \cdot OH + H^+ \quad (1)$$

$$\cdot OH + POBN \longrightarrow POBN\text{-}OH\ (3) \quad (2)$$

The amount of hydroxyl radicals generated, as measured by the intensity of the POBN-OH adduct, is also a function of the vanadyl concentrations as shown in Fig. 2.



(3)

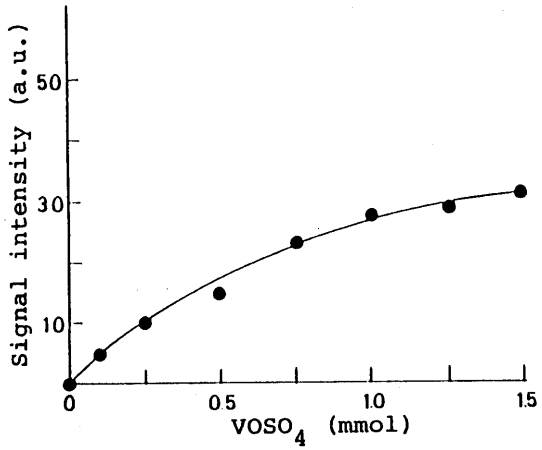


Fig. 2. Hydroxyl Radical Formation from the Reaction of VO^{2+} Ions and H_2O_2
Concentrations: H_2O_2, 10 mmol; POBN, 5 mmol.

Table I. Effect of Hydroxyl Radical Scavenger on the Formation of POBN-OH Adduct during the Reaction of VO^{2+} Ions with H_2O_2

Concentrations: VO^{2+}, 1 mmol; H_2O_2, 10 mmol; POBN, 5 mmol.

Scavenger (mmol)	Inhibition of the formation of POBN-OH adduct (%)
None	0
Ethyl alcohol (100)	100
Dimethyl sulfoxide (100)	100
Thiourea (10)	37
Dithioerythritol (10)	100
dl-Propranolol (10)	45

The formation of hydroxyl radicals during the reaction of VO^{2+} ions with H_2O_2 was also confirmed by finding that the formation of POBN-OH adduct was suppressed or reduced in the presence of hydroxyl radical scavengers[19] as shown in Table I.

Further, the formation of hydroxyl radicals during the reaction of VO^{2+} ions with H_2O_2 was also shown by another spin-trap, DMPO. The characteristic ESR spectrum appeared due to the DMPO-OH adduct ($a^N(1) = a^H(1) = 1.50$ mT).[20]

These results indicate that hydroxyl radicals can be formed by the initial step of the reaction between VO^{2+} and H_2O_2 in an aqueous solution. The mechanism is relevant for biological systems because intracellular vanadium exists exclusively as vanadyl ions, regardless of the oxidation state of the administered vanadium.[21] The hydroxyl radical production of vanadium under physiological conditions may be an important aspect of many of the diverse physiological and toxicological effects of vanadium which are currently unexplained.[12] Intracellular vanadyl ions, either free or bound to macromolecules, may produce hydroxyl radicals in bulk or by a "site-specific" mechanism.[22]

ACKNOWLEDGEMENTS This work was supported in part by a Grant-in-Aid (6357103) for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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(Received January 28, 1989)