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Oxidation of NADH with vanadyl ion: detection of superoxide ion as a reaction intermediate

Toshihiko Ozawa*, Akira Hanaki

Division of Chemical Pharmacology, National Institute of Radiological Sciences, 9-1, Anagawa-4-chome, Chiba-shi 260 (Japan)

and Fumie Takazawa

Kyoritus College of Pharmacy, 1-5-30, Shibakoen, Minato-ku, Tokyo 105 (Japan)

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Abstract

NADH has been not oxidized by the V(V) ion, but by the V(IV) ion. This oxidation occurred in the presence of oxygen, but did not proceed in the presence of superoxide dismutase (SOD) which can dismutate superoxide ion (O_2^-) to oxygen (O_2) and hydrogen peroxide (H_2O_2). Further, the O_2^- spin adduct of α-(4-pyridyl-1-oxide)-*N*-t-butylnitrone (POBN) has been observed during the oxidation of NADH with the V(IV) ion. From these results, it is concluded that O_2^- is generated as a reaction intermediate during the oxidation of NADH by the V(IV) ion in the presence of oxygen.

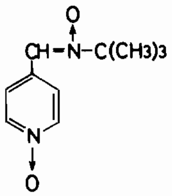
Introduction

It is thought that vanadium is an essential element for living organisms and its ion potentially accelerates the oxidation of NADH in the membranes [1–9]. Recently, the reactivities of vanadate ion (V(V)) [4] and its reducing species, vanadyl ion (V(IV)) [10], towards NADH have been reported independently. However, at present there is no evidence as to which ion is the active species for NADH. We have examined spectroscopically the reaction of the V(IV) ion or the V(V) ion with NADH and the transient intermediate generated from these reactions was investigated. These results are reported in this paper.

Experimental

Reagents

Vanadyl sulfate ($VOSO_4$) was purchased from Aldrich Chemical Co. Ltd. and used without further purification. Ammonium metavanadate (NH_4VO_3) was obtained from Wako Pure Chemical Co. Ltd. NADH, superoxide dismutase (SOD) and catalase were from Sigma Chemical Co. and used as received.



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A water-soluble spin-trap, α-(4-pyridyl-1-oxide)-*N*-t-butylnitrone (POBN, **1**) was from Aldrich Chemical Co. Ltd. and used without further purification. Other reagents were commercially available. Deionized and triply distilled water was used throughout.

Procedure

$VOSO_4$ and NH_4VO_3 were used as the V(IV) ion and V(V) ion, respectively. NADH has the absorption maximum at 340 nm ($\epsilon_{340} = 6.2 \times 10^3$ M^{-1} cm^{-1}) [11], but its oxidized form, NAD^+, does not have a maximum at 340 nm. Thus, the oxidation process of NADH can be followed by measuring the decrease of the absorption at 340 nm. Vanadyl sulfate dissolved in water was stable for several hours, but was not stable for overnight, therefore an aqueous solution of $VOSO_4$ should be freshly prepared.

To detect the short-lived free radicals which may be formed during the oxidation of NADH by vanadium ion, POBN was used as the spin-trap in an aqueous solution.

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*Author to whom correspondence should be addressed.

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All reactants except $VOSO_4$ were dissolved in phosphate buffer at pH 7.4 $VOSO_4$ was dissolved in water.

Reactions were undergone in the phosphate buffer at pH 7.4.

Spectral measurements

The UV–Vis spectrum was measured by a Union Giken SM-401 spectrophotometer. The electron spin resonance (ESR) spectrum was measured by JEOL-PE-1X and JEOL-RE-1X ESR spectrometers (X-band) with 100 kHz field modulation. ESR parameters were calibrated by comparison with a standard Mn^{2+}/MgO and 1,1-diphenyl-2-picrylhydrazyl (DPPH, $g = 2.0036$).

Results and discussion

Addition of V(IV) ion to a solution of NADH in phosphate buffer (pH 7.4) brings about oxidation of NADH (Fig. 1, curve 1). On the other hand, curve 2 shows that the vanadate(V) ion did not enhance the oxidation of NADH. From these results, it is indicated that the active species for the oxidation of NADH is V(IV) ion.

Oxidation of NADH by the V(IV) ion occurred in the presence of oxygen, but not in the absence of oxygen (Fig. 2). Further, the addition of SOD, which disproportionates superoxide ion (O_2^-) to oxygen (O_2) and hydrogen peroxide (H_2O_2), inhibited the oxidation of NADH by the V(IV) ion (Fig. 1, curve 3). However, addition of catalase, which can destroy H_2O_2 to give H_2O and oxygen, did not show any effect on the oxidation of NADH (Fig. 1, curve 4). Further, addition of ethanol, which can trap the hydroxyl radical (·OH), did not inhibit the oxidation of NADH. These results indicate that O_2^- may be formed during the oxidation of NADH in the presence of oxygen.

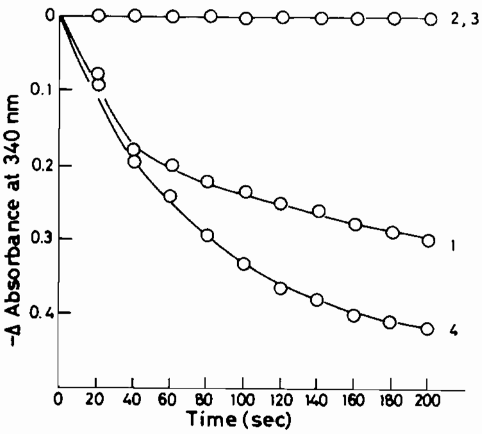


Fig. 1. Oxidation of NADH by V(IV) and V(V) ions in the presence of oxygen. Curve 1: 0.83 mM NADH + 0.34 mM V(IV); curve 2: 0.83 mM NADH + 0.34 mM V(V); curve 3: 0.83 mM NADH + 0.34 mM V(IV) + 0.017 mg/ml SOD; curve 4: 0.83 mM NADH + 0.34 mM V(IV) + 0.017 mg/ml catalase.

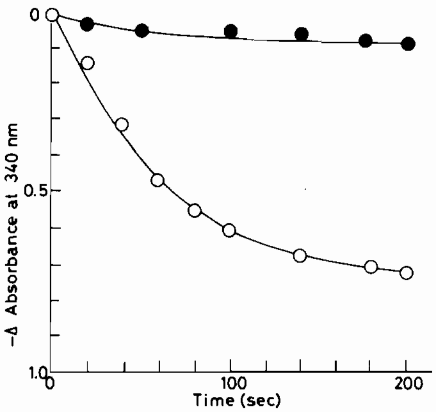


Fig. 2. Oxidation of NADH by V(IV) ion in the presence (○) and in the absence (●) of oxygen. Reaction mixtures contained 1.00 mM NADH and 0.25 mM V(IV) at pH 7.4.

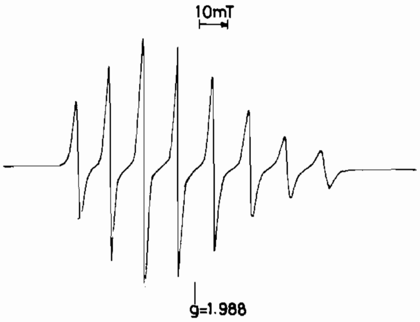


Fig. 3. ESR spectrum observed from V(IV) ion in aqueous solution. ESR settings: microwave power, 10 mW; modulation amplitude, 0.07 mT; amplitude, 3.6 × 100; time constant, 0.3 s; scan time, 8 min.

Furthermore, to ascertain the formation of O_2^- during the oxidation of NADH by the V(IV) ion, ESR spin-trapping experiments were carried out. When the ESR spectrum was measured immediately after the V(IV) ion (0.2 mM) had been mixed with NADH (5 mM), the ESR spectrum due to the V(IV) ion ($I = 7/2$) consisting of 8 lines ($a^{V(IV)} = 11.61$ mT, $g = 1.998$) as shown in Fig. 3 completely disappeared, but no new signal due to a secondary radical was observed. However, when POBN was added to the aqueous solution of NADH before the two reactants were mixed, a doublet of triplet ESR signal was observed as shown in Fig. 4(a). This signal can be analyzed as follows: $a^N(1) = 1.51$ mT, $a^H(2) = 0.17$ mT and $g = 2.004$. Addition of SOD to the NADH solution before the V(IV) ion was mixed caused the

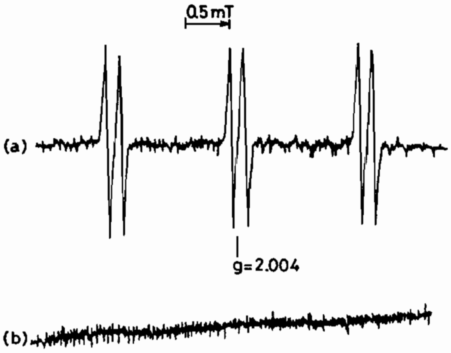
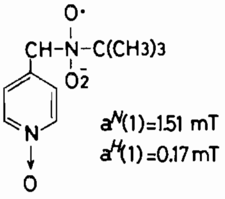


Fig. 4. ESR spectrum observed during the reaction of V(IV) ion with NADH in the presence of POBN: (a) without SOD; (b) with SOD (0.017 mg/ml). Concentrations: V(IV), 0.2 mM; NADH, 5 mM. ESR settings: same as Fig. 3.

disappearance of this ESR signal as shown in Fig. 4(b). Further, this ESR signal is different from that of the POBN adduct of the OH radical ($a^N(1) = 1.50$ mT, $a^H(2) = 0.15$ mT and $g = 2.004$) which is obtained by the reaction of the V(IV) ion with H_2O_2 [12]. From these results, the ESR signal shown in Fig. 4(a) can be assigned to the POBN adduct of O_2^- (2).



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From the results mentioned above, the following reaction mechanism may be assumed.

$$V(IV) + O_2 \longrightarrow V(V) + O_2^- \qquad (1)$$

$$V(V) + O_2^- \longrightarrow V(V){-}O_2^- \qquad (2)$$

$$V(V){-}O_2^- + NADH + O_2 \longrightarrow V(IV){-}OOH + NAD^+ + O_2^- \qquad (3)$$

$$O_2^- + POBN \longrightarrow POBN{-}O_2^- \ (2) \qquad (4)$$

Since the formation of the $V(V){-}O_2^-$ adduct has already been determined by the reaction of vanadyl ion with H_2O_2 by use of the ESR rapid-mixing flow technique [13–15], we are now trying to detect the $V(V){-}O_2^-$ complex.

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