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Formation of an oxo-radical of peroxovanadate during reduction of diperoxovanadate with vanadyl sulfate or ferrous sulfate

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Abstract

Formation of oxygen radicals during reduction of H_2O_2 or diperoxovanadate with vanadyl sulfate or ferrous sulfate was indicated by the 1:2:2:1 electron spin resonance (ESR) signals of the DMPO adduct typical of standard $^{\bullet}OH$ radical. Signals derived from diperoxovanadate remained unchanged in the presence of ethanol in contrast to those from H_2O_2. This gave the clue that they represent a different radical, possibly $^{\bullet}OV(O_2)^{2+}$, formed on breaking a peroxo-bridge of diperoxovanadate complex. The above reaction mixtures evolved dioxygen or, when NADH was present, oxidized it rapidly which was accompanied by consumption of dioxygen. Operation of a cycle of peroxovanadates including this new radical is suggested to explain these redox activities both with vanadyl and ferrous sulfates. It can be triggered by ferrous ions released from cellular stores in the presence of catalytic amounts of peroxovanadates.

Keywords: Peroxovanadate cycle; Diperoxovanadate; Vanadyl; $^{\bullet}OV(O_2)^{2+}$ radical

1. Introduction

Among the diverse cellular phenomena associated with H_2O_2 formed in small quantities in all tissues [1] are respiratory burst, egg fertilization, parasite maturation, hormonal response, membrane transport, cyanide-resistant respiration, cellular thermogenesis and lignification [2]. Several intracellular components and enzymes are directly affected upon addition of H_2O_2 [3] and the number is increasing. H_2O_2, to be effective, must be converted into a stable form in the presence of abundant catalase and glutathione peroxidase and still capable of peroxidation reaction. Also present in small quantities in cells, vanadate readily forms peroxo-complexes with H_2O_2 of the types VOOH (monodentate), $V(O_2)$ (bidentate) and VOOV (μ-peroxo) and other derivative radicals. These can diversify redox capabilities of both vanadate and H_2O_2 and therefore are of interest to study.

During oxidation of cationic vanadyl (VO^{2+}) by H_2O_2 in acid medium, formation of an addition complex, monoperoxovanadate ($OVOOH^{+}$), followed by breaking of its unstable peroxo-bridge yielding vanadate (OVO^{+}) and $^{\bullet}OH$ radical was reported in a classic paper by Brooks and Sicilio [4]. A multi-banded spectrum in kinetic electron spin resonance (ESR) studies of the reaction mixture indicated the formation of a new radical. The authors identified it as $OVOO^{2+\bullet}$, a peroxovanadium radical derived from peroxovanadate on oxidation by $^{\bullet}OH$. Release of dioxygen that accompanies these reactions was ascribed to dismutation of this radical.

Jaswal and Tracey [5] presented extensive data on formation and decomposition under conditions of higher pH of peroxovanadates identified by their characteristic chemical shifts in ^{51}V-NMR spectra. Diperoxovanadate

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(DPV) was found to be the most stable species at neutral pH. It fits the role of active cellular peroxide as it is 50 times more stable to degradation by catalase and substitutes 100 times more effectively in peroxidase reactions than H_2O_2 [6]. The crystal structure of ammonium DPV [7] identified it to be a dimer in solid state corresponding to $O[OV(O_2)_2]_2$. Interestingly, each vanadium atom has an unusual seventh coordination with a bidentate oxygen atom of the other V-atom. A query was raised whether monomerization leads to V-OO of one of the bidentate O–O [8]. Jaswal and Tracey [5] used the formula $H_2VO_2\,(OO)_2^-$ to represent DPV which can exist either as O^- or OO^-. The linear form will be needed as in HOOH for complexing with vanadyl and redox activity.

These findings served as resource information for our studies on DPV-dependent oxidation of vanadyl [9,10], NADH [11] and bromide [12]. Applying these principles for the reactions of vanadyl with DPV, we proposed that μ-peroxo-bridged mixed valency divanadate $[OVOOV(O_2)]^{3+}$, an addition complex similar to $OVOOH^+$, is the corresponding active intermediate [11,13]. Such a bridged divanadate was reported in Ref. [14]. The expected product of breakdown of its peroxo-bridge is the radical $^{\bullet}OV(O_2)^{2+}$. The ESR spectrum of dimethyl pyrroline-*N*-oxide (DMPO) adduct and its properties supported its presence [10]. We now reproduce these and show that Fe^{2+} substitutes for OV^{2+} in these reactions with DPV.

2. Materials and methods

NADH, superoxide dismutase (SOD), catalase, DMPO, and other biochemicals mentioned were obtained from Sigma Chem. (St. Louis, MO). A sample of analytical grade vanadyl sulfate, obtained from S.D. Fine Chemicals (Mumbai, India), was recrystallized from aqueous solution, and concentration of its stock solution was determined by absorbance at 750 nm (0.018 for 1 mM). Potassium mono- and di-hydrogen phosphates and H_2O_2 (30%, w/v) were obtained from BDH (Mumbai, India). A stock solution of H_2O_2 was standardized by its absorbance at 280 nm (0.042 for 1 mM). The solutions were made fresh in water doubly distilled in a quartz apparatus.

Diperoxovanadate $[KOV(O_2)_2 \cdot H_2O]$ was prepared from vanadium pentoxide and H_2O_2 as described earlier [15]. An aqueous solution of this preparation in 50 mM phosphate buffer (pH 7.0) showed a single major peak at −705 ppm in the ^{51}NMR spectrum.

The standard reaction mixture contained phosphate buffer (50 mM, pH 7.0), H_2O_2 (0.2 mM) and vanadyl sulfate (0.2 mM) for oxygen release experiments. Where mentioned, the following replacements were done: DPV (0.2 mM) for H_2O_2 and ferrous ammonium sulfate (0.2 mM) for vanadyl sulfate. In addition, NADH (0.2 mM) was included before others in the buffer, in the experiments on NADH oxidation and accompanying oxygen consumption. The reaction was started by adding vanadyl (or ferrous) and was monitored at 30 °C.

Measurements of release and consumption of oxygen were carried in a Gilson 5/6 H oxygraph fitted with a Clark electrode in a reaction vessel (2.2 ml). Setting the recorder pen in the middle of the chart, the machine was standardized by increase in dissolved oxygen with excess catalase (0.1 mg protein) added to solutions containing known amounts of H_2O_2. The value of 224 μM was used for the concentration of dissolved oxygen under these conditions. Oxidation of NADH was measured in identical reaction mixtures by the decrease in absorbance at 340 nm ($\Delta A_{340\ nm}$) in a Shimadzu double-beam spectrophotometer.

The ESR spectra of aqueous solutions were recorded at room temperature in a capillary tube in a Varian Model E spectrometer. The following conditions were employed: microwave power, 5 mW; microwave frequency, 9.05 GHz; modulation frequency, 100 kHz; modulation amplitude, 0.5×1 G; time constant 0.064 s; scan time, 2 min; scan range, 2×100 G; field set 3220×G, and receiver gain 1.25×10^3. The reaction mixtures contained phosphate buffer (50 mM, pH 7.0) and DMPO (100 mM), with or without ethanol (1.7 M). H_2O_2 or DPV and vanadyl or ferrous were added in that order at concentrations given in the legend. The spectra were recorded within 2 min of mixing the reactants.

Phosphate buffer is the best for detecting $^{\bullet}OH$ radicals and used widely wherever it does not interfere by binding to metal reactants [16]. Phosphate does bind to vanadium compounds occupying at least one coordination site. In the present experiments, its presence in mM concentrations is essential for obtaining maximal rates. Therefore, 50 mM phosphate buffer was included in the above reaction mixtures.

3. Results

The well-known standard system of a ferrous salt and H_2O_2 to generate $^{\bullet}OH$ radicals was chosen for this study. We studied the effects of replacing ferrous sulfate with vanadyl sulfate, and H_2O_2 with DPV. Combinations of these four components were tested for radical generation, oxygen release and NADH oxidation.

3.1. Generation of oxo-radicals during oxidation of ferrous and vanadyl compounds

Addition of ferrous ammonium sulfate to a solution containing phosphate buffer, DMPO and H_2O_2 showed good 1:2:2:1 quartet signal ($\alpha_N=\alpha_H=14.9$ G) of the adduct DMPO-OH (Fig. 1, line 1). Similarly, addition of vanadyl sulfate to phosphate buffered mixture of DMPO and H_2O_2, also known to generate $^{\bullet}OH$ radicals under these conditions [17,18], showed the same signal of the adduct (Fig. 1, line 2). On inclusion of ethanol before adding ferrous and vandyl