ANTIOXIDANT AND FREE RADICAL SCAEVGING ACTIVITY OF A VANADUIM COMPLEX.

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Summery

Vanadium is a trace metal in biological system with insulin-like action and anticancer activity. The potential benefit of vanadium complexes as oral insulin substitutes for the treatment of diabetes have been investigated. This study was designed to evaluate the in vitro antioxidant capacity of a vanadium complex using different in vitro antioxidant tests, including superoxide radicals and hydrogen peroxide scavenging and inhibitory effect on protein oxidation as well as the inhibition of Fe^{2+}/ascorbate induced lipid peroxidation in rat liver homogenate. The results revealed that the vanadium complex dose-dependently scavenges superoxide radicals and hydrogen peroxide. This compound has also notable inhibitory effects on protein oxidation and capable to prevent the formation of thiobarbituric reactive substances in Fe^{2+}/ascorbate-induced lipid peroxidation in rat liver homogenate.

Keywords: Vanadium; Oxidative stress; Lipid peroxidation; Protein oxidation.

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Introduction

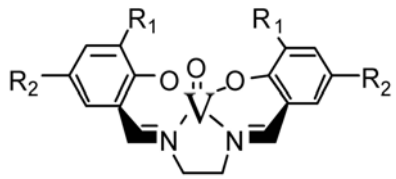
Molecular oxygen plays a pivotal role in cellular respiration and is essential for maintaining high-energy metabolic processes. However, it can be also toxic. The reduction of molecular oxygen can produce superoxide radicals ($O_2^{-\bullet}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2), that are the major species of reactive oxygen species (ROS). ROS is reported to exert some destructive effects, including lipid peroxidation, protein oxidation, enzymes inactivation and DNA breakage [1] and has been implicated in initiating and/or causing pathogenesis of some diseases [2]. Oxidative stress is defined in general as excess formation and/or incomplete removal of ROS [3, 4]. Cells have several antioxidant defense mechanisms that help to prevent the destructive effects of ROS [5]. The efficiency of the antioxidant defense system is altered under pathological conditions and therefore, the ineffective scavenging and/or overproduction of ROS may play a crucial role in determining tissue damages [6, 7]. In oxidative stress condition, ROS can induce lipid peroxidation in the cellular biomembrane that can affect the membrane structure and its function. On other hand, lipid peroxidation products such as malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) can react with biomolecules and exert cytotoxic and genotoxic effects. The high levels of lipid peroxidation products have been found in some diseases condition such as liver disease, diabetes, vascular disorders and tumors [8]. Antioxidants are reported to prevent oxidative damages and play an important role in preventing and/or treatment of the chronic diseases by reducing the destructive effects of ROS [9, 10]. In an effort to prevent and/or diminish ROS destructive effect, investigators have evaluated compounds that scavenge ROS and reduce ROS-induced damages.Vanaduim is a trace metal in biological system that have insulin-like action [11, 12] and anticancer activity [13]. The potential benefits of vanadium compounds as oral insulin substitutes for the treatment of diabetes have been investigated [14, 15].

However, in the present study, we evaluated the antioxidant and free radicals scavenging activity of a vanadium complex in different in vitro antioxidant assay systems. Based on the results obtained in this study vanadium complex possess a high antioxidative and free radical scavenging activity in all in vitro systems used to investigate the subject matter.

Materials and Methods

Chemicals

Ascorbic acid, 6-hydroxy-2, 5, 7, 8-tetra methyl chroman-2-carboxylic acid (Trolox), tricholoroacetic acid (TCA) and ferric chloride were obtained from Sigma (Sigma-Aldrich, Sternheim, Germany). Reduced nicotinamide adenine dinucleotide (NADH), phenazine methosulphate (PMS), nitroblue tetrazolium (NBT), hydrogen peroxide (H_2O_2), 2-deoxy-2-ribose, butylated hydroxyl toluene (BHT) and 2-thiobarbituric acid (TBA) were obtained from Merck (Germany). Salicylaldehyde and ethylenediamine were purchased from Fluka (Buchs, Switzerland). All other chemicals used were analytical grade and obtained from either Sigma-Aldrich or Merck (Germany). The synthesis and purification of vanadium complex (scheme I) was achieved as reported previously [16].



R_1=H, R_2= OMe

Scheme 1. Chemical structure of vanadium complex

Hydrogen peroxide scavenging activity

The hydrogen peroxide scavenging activity of vanadium complex was studied according to the slightly modified

method of Ruch et al [17]. Different concentration of the vanadium complex was added to H_2O_2 solution (2ml, 10 mM) in phosphate buffer (PH 7.4, 50 mM) and the reaction mixture was incubated at 25 °C for 30 min. The unreacted H_2O_2 was determined by measuring the absorbance value of the reaction mixture at 230 nm with respect to the blank solution (phosphate buffer plus the vanadium complex without H_2O_2). The percentage of hydrogen peroxide scavenging activity of the vanadium complex was calculated using the following equation:

H_2O_2 scavenging activity (%) = $[(A-A_1/ A) \times 100]$ where A is the absorbance of the control and A_1 is the absorbance in the presence of the vanadium complex.

Scavenging of superoxide anion in PMS-NADH-NBT system

Superoxide anion scavenging activity of the vanadium complex was determined based on the method described by Liu [18]. In PMS-NADH/ NBT system, superoxide anions are generated as a result of NADH oxidation which is then assayed through NBT reduction. In our experiment, superoxide anions were generated in 3 ml of Tris-HCl buffer (16 mM, PH 8.) containing 1 ml of NBT (50 μM), 1ml NADH (78 μM) and the sample solution of the vanadium complex. The vanadium complex has been dissolved in a minimum amount of DMSO. The DMSO concentration in each reaction mixture has been restricted to 0.1% and this concentration has been present in all vehicle solutions. The reaction mixture was started by adding 1 ml of PMS solution (10 μM) to the mixture and after 5 min incubation at 25 °C, the absorbance was measured at 560 nm against the corresponding blank. Based on principle of the technique, the decrease in the absorbance correlates with increase superoxide anion scavenging activity. The percentage of superoxide anion scavenging activity was calculated using the following equation:

$O_2^{-\bullet}$ scavenging activity (%) = $[(A-A_1/ A) \times 100]$, where A is the absorbance of the control, and A_1 is the absorbance of the vanadium complex.

Antioxidant assay using rat liver homogenate

The assay is based on the ability of antioxidants toward the inhibition of lipid peroxidation in rat liver homogenate in presence of $FeSO_4$ and ascorbate. Male wistar rats, weighing 250-320 g/each, received food and water ad libitum.The investigation was performed in accordance with guidelines for care and use of experimental animals approved by state veterinary administration of University of Tehran. All animals were anaesthetized with diethyl ether and their liver was removed and homogenized in phosphate buffer (50 mM PH 7.4) to give a 10% (W/V) liver homogenate. The liver homogenate was further centrifuged at 5000 g for 15 min. The supernatant was collected and its protein content was measured using Lowry's method [19]. The extent of lipid peroxidation in the presence and absence of the vanadium complex was evaluated based on the extent of thiobarbituric acid reactive substances (TBARS) [20]. In this assay, Trolox was used as the positive control.

Inhibition of protein oxidation in H_2O_2/Fe^{3+}/ascorbate model

The inhibitory effect of vanadium complex on protein oxidation induced by H_2O_2/Fe^{3+}/ascorbate was determined according to the method of Wang et al [21]. In this test, bovine serum albumin (BSA) was incubated with H_2O_2, $FeCl_3$ and ascorbic acid in presence of different concentrations of the vanadium complex for 30 min and the protein carbonyl formation (PCO) was measured based on the extent of absorbance of each reaction mixture at 370 nm and the inhibitory effect of the vanadium complex was expressed as percentage of inhibition relative to the control sample.

Statistical analyses

All data are presented as means ± S.D. The mean values were calculated based on the data taken from at least three independent duplicate experiments conducted on separate days using freshly prepared reagents

Results and discussion

There are numerous methods to evaluate the antioxidant activity of various compounds. Among these methods, reducing power, metal chelation and free radical scavenging activities are the most commonly used approaches [22]. In this study, we selected H_2O_2 and $O_2^{-\bullet}$ scavenging, lipid peroxidation and protein oxidation assays to evaluate the antioxidant capacity of the vanadium complex.

Hydrogen peroxide scavenging activity

Hydrogen peroxide is a non-radical form of ROS that is formed in living organisms by superoxide dismutase. Hydrogen peroxide is not by itself very active but it can cross biological membranes and generates hydroxyl radicals which are toxic to cells and can damage a number of biomoleucles [23]. Thus, removing of H_2O_2 is very important for protection of living organism. The ability of the vanadium complex to scavenge H_2O_2 is shown in Fig.1.

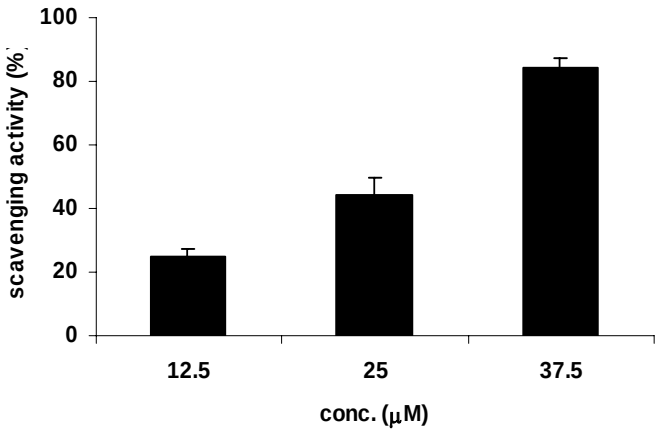


Figure 1. Hydrogen peroxide scavenging activity of vanadium complex. Each value represents the mean ± SD (n=3).

As shown in Fig.1, the vanadium complex exhibits strong H_2O_2 scavenging activity with IC_{50} value of 24.2 μM.

Superoxide anion scavenging activity

Superoxide anions are usually generated as a result of various biological reactions. These anions can be converted to hydrogen peroxide and finally to hydroxyl radicals [24]. Compared to other oxygen radicals, superoxide anions have longer half lifes with potential damaging effects on biomolecules mainly lipids. They can damage biomolecules or initiate lipid peroxidation during various patho-physiological events [25, 26].

In PMS-NADH/NBT system, the $O_2^{-\bullet}$ anions generated from dissolved oxygen by PMS-NADH coupling reaction, will reduce NBT. The progress of the reaction is followed by measuring the absorbance at 560 nm [27]. In the presence of an antioxidant, the extent of absorbance at 560 nm will decrease. As shown in Fig.2, the vanadium complex inhibited the reduction of NBT in a dose-dependent manner. The IC_{50} value of this compound is 29 μM. These results clearly indicated that the vanadium complex is a potent scavenger of superoxide radicals in a dose-dependent manner.

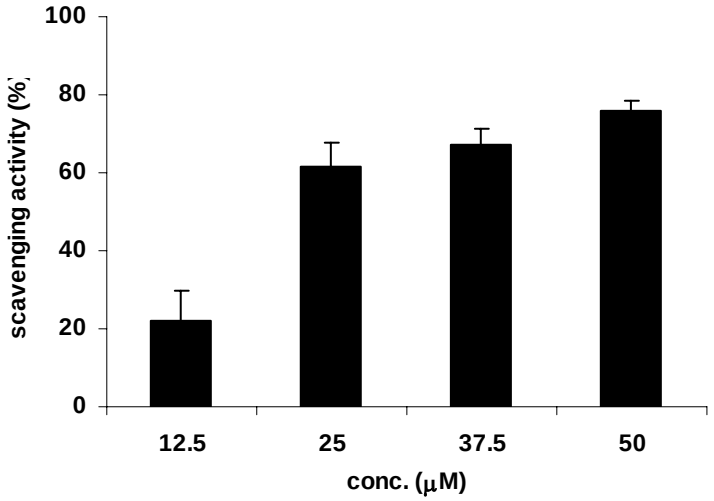


Figure 2. Superoxide radical scavenging activity of vanadium complex. Each value represents the mean ± SD (n=3).

Inhibition of lipid peroxidation in rat liver homogenate

Peroxidation of polyunsaturated fatty acids of the cell membranes can produce chemically reactive products which can react with protein and DNA content of the cell. The most abundant lipid peroxidation product is malondialdehyde (MDA) which can react and cross-link different biological molecules. Based on this fact, MDA is usually regarded as a marker of oxidative stress [27]. Lipid peroxidation is involved in numerous pathological events [28]. Thus, inhibition of lipid peroxidation can protect living organism against oxidative stress. The addition of FeSO4-ascorbic acid to the liver homogenate for 30 min significantly increased the extent of TBARS formation, compared to the control sample (4.31 nmol/mg protein versus 0.28 nmol/mg protein).

Table 1:Effect of vanadium complex and Trolox on Fe^{2+} / ascorbate induced lipid peroxidation in rat liver homogenate

Concentration (μM)	TBARS (nmol/mg protein)	Inhibition (%)
Fe^{2+}/ascorbate[a]	4.31 ± 0.071	-
Control[b]	0.28 ± 0.023	-
0.25	3.76 ± 0.098	14.86 ± 0.87
0.5	3.21 ± 0.035	25.35 ± 2.02
0.75	1.48 ± 0.141	65.64 ± 2.78
1	0.59 ± 0.035	86.22 ± 0.95
1.25	0.26 ± 0.072	93.68 ± 1.63
Trolox (400 μM)	0.26 ± 0.024	94.06 ± 0.43

Each value represents the mean ± SD (n = 3). All values statistically different.
[a] Reaction mixture consisted of Fe^{2+} / ascorbate
[b]Reaction mixture without Fe^{2+} / ascorbate

However, as shown in Table 1, adding 0.25-1.25 μM of the vanadium complex to rat liver homogenate significantly reduced TBARS formation in the liver homogenate, indicating significant anti-lipid peroxidation activity of the vanadium complex. This compound was capable of inhibiting TBARS formation by 14.86%, 25.35%, 65.64%, 86.22% and 93.68% at the vanadium complex concentrations of 0.25, 0.5, 0.75, 1 and 1.25 μM respectively.

Inhibition of protein oxidation

Free radical-mediated damages of proteins are mainly initiated by metal-ion dependent reactions and auto-oxidation of sugers and lipids [29]. In cells, the oxidized proteins are usually handled by proteinases. However, some oxidized proteins are poorly handled by the affected cells and therefore, they might constitute the pathological background of some health disorders such as atherosclerosis, diabetes and neurodegenerative diseases [29]. Protein oxidation is measured in terms of protein carbonyl content. As shown in Fig.4, the vanadium complex dose-dependently exhibited inhibitory effects on PCO formation.

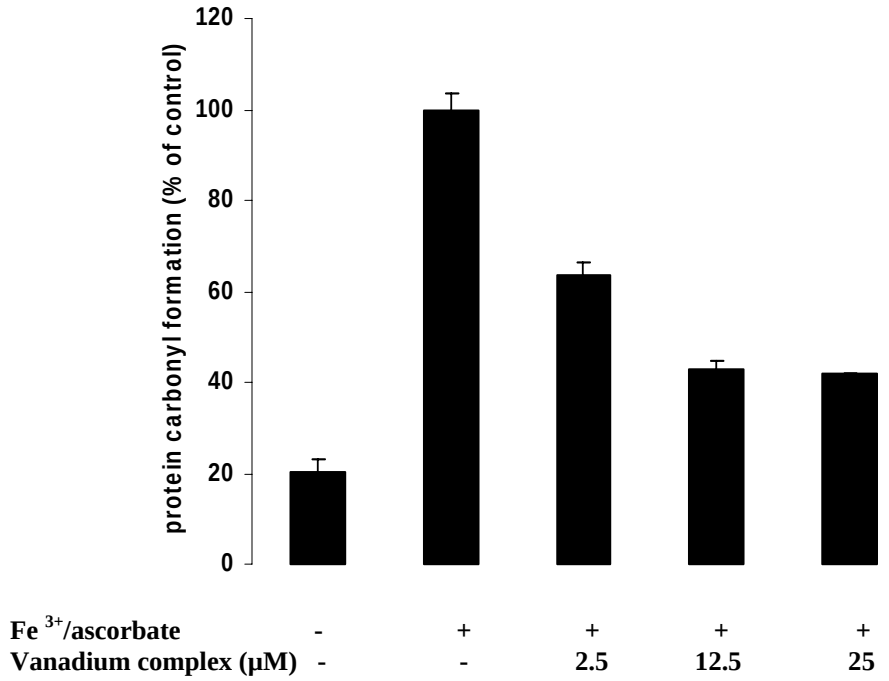


Figure 4. Inhibitory effect of the vanadium complex on protein (BSA) oxidation expressed as protein carbonyl formation (PCO) induced by H_2O_2/Fe^{3+}/ascorbate system. Each value represents the mean ± SD (n=3). All values statistically different ($P < 0.05$).

Conclusion

In this study, using various in vitro assay systems, the antioxidant activity of the vanadium complex was evaluated based on superoxide and hydrogen peroxide scavenging activities, inhibition of lipid peroxidation in rat liver homogenate. In addition, we further evaluated the inhibition effect of this compound on protein oxidation. The results clearly confirmed the antioxidative and free radical scavenging activity of the vanadium complex.

Acknowledgments

The authors appreciate the financial support of this investigation by the Research Council of University of Tehran.

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