

Reactive Oxygen Species Scavenging by Small Gadolinium-Yttrium Orthovanadate Nanocrystals

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Abstract — Oxidative stress induced by reactive oxygen species (hydrogen peroxide, superoxide anion, hydroxyl radical etc.) is the key factor in developing a variety of pathological conditions including diabetes, asthma, atherosclerosis, hypertension mellitus and cancer. To prevent or reduce the cell damages caused by reactive oxygen species, natural or synthetic molecules named antioxidants are commonly used. Recently, various inorganic nanomaterials, such as CeO₂, Mn₃O₄, Co₃O₄ and Fe₃O₄ nanocrystals, have attracted growing interest as nano-antioxidants with reactive oxygen species regulating ability.

Here, we report on high antioxidant behavior of small gadolinium-yttrium orthovanadate nanocrystals revealed by spectroscopic methods using various reactive oxygen species sensors, and propose the mechanism describing their reactive oxygen species scavenging action.

It has been found that in 2 nm gadolinium-yttrium orthovanadate nanocrystals more than 60% vanadium ions are presented in lower V⁴⁺ and V³⁺ oxidation states. Switching between V³⁺ ↔ V⁴⁺ and V⁴⁺ ↔ V⁵⁺ oxidation states allow gadolinium-yttrium orthovanadate nanocrystals to react with hydrogen peroxide (H₂O₂) and superoxide anion (O₂^{•-}) in enzyme-like manner and neutralize hydroxyl radical (•OH), and peroxy radicals (ROO•), as well.

The obtained data and proposed mechanism of gadolinium-yttrium orthovanadate nanocrystals antioxidant action can explain the non-trivial anti-aging and radioprotective effects of

gadolinium-yttrium orthovanadate nanocrystals observed earlier *in vivo*.

Keywords — reactive oxygen species, nano-antioxidants, orthovanadate nanocrystals

I. INTRODUCTION

Oxidative stress is a state characterized by the imbalance between the formation of oxidants (reactive oxygen species – ROS, or reactive nitrogen species – RNS) and the cell's effective antioxidant response [1-3]. Stresses, hypercaloric diet and sedentary lifestyle trigger the overproduction of ROS (hydrogen peroxide – H₂O₂, superoxide anions – O₂^{•-}, hydroxyl radicals – •OH) which are highly toxic and provoke irreversible damages or even cell death [1-3]. Moreover, oxidative stress is considered to contribute to a variety of pathological diseases (atherosclerosis, hypertension, cancer, neurological disorders, diabetes mellitus, asthma etc.) [1-3]. Natural or synthetic antioxidants can prevent oxidative stress caused by ROS. However, malabsorption, difficulty in crossing the cell membranes, and degradation during delivery are the main challenges that limit their bioavailability.

Recently, different types of nanosized materials (carbon nanotubes, fullerenes, metal and metal oxide nanoparticles (NPs), polymer-loaded antioxidant NPs) have attracted growing interest as nano-antioxidants [3-5]. Many

nanoparticles are good radical scavengers in aqueous systems due to the presence on their surface of electron-accepting and donating sites, which may be either inherent such as in CeO₂ [6] and in Pt nanoparticles [5] or formed by UV-irradiation, as in case of TiO₂ and ZnO nanoparticles [5].

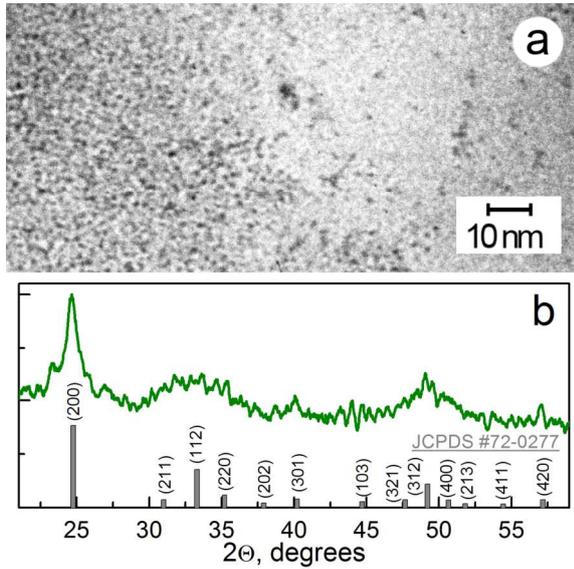


Fig. 1. TEM image (a) and XRD pattern (b) of synthesized orthovanadate nanocrystals.

Antioxidant activity of orthovanadate nanocrystals is reported to be the main factor increasing the reliability of the ROS control system in tissues and especially in the liver mitochondria of old animals [7-8]. Moreover, radioprotective effects of orthovanadate nanocrystals at X-ray irradiation has been also described earlier [9-11]. Here, we report on high antioxidant behavior of small gadolinium-yttrium orthovanadate nanocrystals revealed by spectroscopic methods using various reactive oxygen species sensors, and propose the mechanism describing their reactive oxygen species scavenging action.

II. MATERIALS AND METHODS

A. Characterization of Nanocrystals

Gadolinium-yttrium orthovanadate nanocrystals synthesized by the colloidal method reported by us earlier [10] were extra-small of about 2 nm size and spherical form, which was confirmed by transmission electron microscopy (TEM) characterization (Fig. 1a). Their crystalline tetrahedral zircon-type structure were confirmed by X-ray powder diffraction analysis (Fig. 1b) with a pattern matching well to the standard diffraction data for bulk orthovanadate crystal with tetrahedral zircon-type structure (JCPDS, no. 72-0277).

To verify the valence state of vanadium ions in obtained gadolinium-yttrium orthovanadate nanocrystals, X-ray photoelectron spectroscopy (XPS) was used. The XPS pattern for obtained nanocrystals is shown in Fig. 2. The core level spectra of V2p for obtained nanocrystals and the peak positions are in a good agreement with those reported on other vanadate compounds [12-13]. However, a careful analysis of the V2p_{3/2} band (518 eV) reveals an asymmetry at the lower binding energy site that indicates the presence of vanadium ions in more than one oxidation state. The band is fitted well with three Gaussian-Lorentzian peaks with

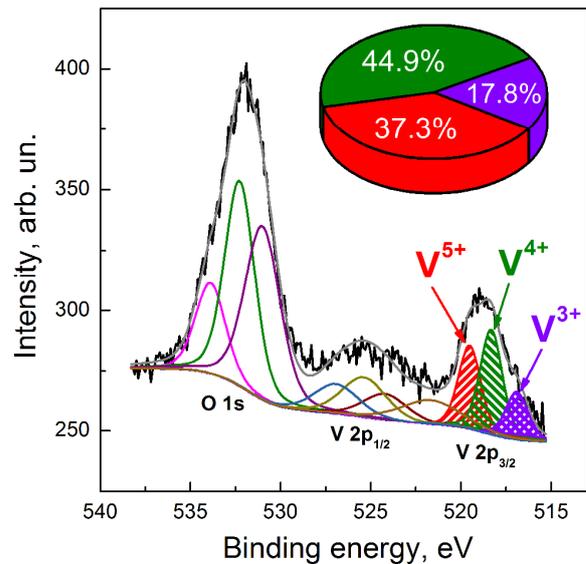


Fig. 2. XPS pattern of synthesized orthovanadate nanocrystals (inset – relative concentrations of V³⁺, V⁴⁺ and V⁵⁺ ions).

BE = 516.9, 518.5 and 519.5 eV, which are associated with V³⁺, V⁴⁺ and V⁵⁺, respectively, and are in good agreement with the literature data [12]. The calculated relative concentrations of the vanadium ions in obtained gadolinium-yttrium orthovanadate nanocrystals are 17.8% (V³⁺), 44.9% (V⁴⁺) and 37.3% (V⁵⁺) (Fig. 2 - inset). Obtained results show that in small gadolinium-yttrium orthovanadate nanocrystals more than 60% of vanadium ions on the surface of NPs are in lower oxidation states (V⁴⁺ and V³⁺). The coexistence of three oxidation states of vanadium ions in obtained nanocrystals is promising for the consideration of this material as a potential free radical scavenger with antioxidant properties.

B. Antioxidant Activity Study

O₂-• scavenging ability. To estimate the ability of NPs to scavenge O₂-•, the method based on the step-wise reaction of epinephrine (adrenaline) autoxidation to adrenochrome with production of O₂-• as an intermediate product, was used [14]. At high pH, epinephrine is quickly oxidized to adrenochrome that could be detected by appearance of new fluorescence band at an analytical wavelength of 510 nm. Experimental procedure was as follows. 30 μL of 0.18% solution of epinephrine hydrochloride was added in the NPs (0.1 gL⁻¹) borate buffer solution (pH=10.6). The solution was stored in the dark to start the epinephrine oxidation and fluorescence spectra were recorded at different time intervals with a spectrofluorimeter Lumina (ThermoScientific, USA). Fluorescence intensity at λ_{max}=510 nm associated with the fluorescence oxidative product adrenochrome was analyzed. As a control, borate buffer solution (pH=10.6) without NPs was used.

DPPP oxidation test. To estimate the ability of NPs to decompose H₂O₂ in aqueous solutions, a fluorogenic reagent DPPP was used [15]. DPPP is essentially nonfluorescent until oxidized to a phosphine oxide by peroxides that can be detected spectrofluorometrically by the appearance of new band with the main maximum at 380 nm. The experimental procedure was the following. 600 μL of H₂O₂ (1M) were added to 10 mL of the NPs colloidal solution (0.1 gL⁻¹) and then kept in the dark. H₂O₂ decomposition in the presence of NPs was analyzed. At different time

intervals, 980 μL of the mixed solution was pipetted to a quartz cuvette and 20 μL of the DPPP solution in DMSO (1mM) was added. The obtained solution was kept for about 30 min for completing DPPP reaction with H_2O_2 , and then a fluorescence spectrum was taken using a Lumina spectrofluorimeter (Thermo Scientific, USA) with the excitation at 350 nm. As a control, a water solution containing the same amount of H_2O_2 , but without NPs was used.

OH scavenging ability. For detection of the $\cdot\text{OH}$ generation efficiency in the solutions with/without NPs, $\cdot\text{OH}$ specific probe coumarin was used [16]. The route of the $\cdot\text{OH}$ detection is based on the formation in a solution of fluorescent product of the coumarin oxidation (7-hydroxycoumarin) detected by a new fluorescent band with a maximum at 460 nm. The experimental procedure was the following. The required amount of the coumarin was mixed with the colloidal NPs solutions to reach final coumarin and NPs concentrations of 0.1 mmolL⁻¹ and 1 gL⁻¹, respectively. Obtained solutions containing NPs and coumarin were placed in plastic cuvettes (10x10 mm) and irradiated from the open part by X-ray using an ISOVOLT-160 TitanE device with a tungsten anode (30kV, I=20 mA). The distance from the X-ray tube to the irradiated samples was 25 cm.

Assessment of lipid oxidation. Effects of NPs on lipid autoxidation dynamics were studied using a PC liposomes suspension. To estimate conjugated dienes formation (the product of lipid oxidation), 100 μL of the PC liposome suspension (10⁻³ M) was mixed with 100 μL of NPs water solution (0.2 g L⁻¹) and 800 μL of bi-distilled water was added. For lipid autoxidation test, the obtained PC-NPs suspensions were kept in a heat chamber ($t=65^\circ\text{C}$) for different time intervals. The relative concentration of conjugated dienes formed in a water solution was estimated by measuring the absorbance of the suspensions at characteristic wavelength 234 nm (conjugated dienes maximum) [17]. As a control, the suspension without NPs was taken.

III. RESULTS AND DISCUSSION

The presence of vanadium ions in lower valence states, i.e. V^{3+} and V^{4+} , indicates that obtained NPs could act as a reductant donating electron in redox reactions. To verify $\text{O}_2^{\cdot-}$ and H_2O_2 neutralization abilities of NPs in aqueous solutions, we use specific sensors. $\text{O}_2^{\cdot-}$ anions are generated in the solution containing adrenaline at high pH (pH=10.6) and provokes its oxidation with a fluorescent product adrenochrome formation [14]. Fig. 3 shows that in the solution containing NPs adrenaline oxidation is remarkably slowed down indicating the sufficient decrease in the $\text{O}_2^{\cdot-}$ level in the solution as compared with the control. Such $\text{O}_2^{\cdot-}$ scavenging activity was recently reported for a number of oxide nanoparticles [5].

Fig. 4 shows that NPs efficiently decompose hydrogen peroxide (H_2O_2) in water solution. In our experiment NPs (0.1 g/L) placed in 10 ml cuvettes decomposes ~47 % of 6mM H_2O_2 in 24 h or 20.83 nmol min⁻¹. Assuming NPs as spheres with $d=2$ nm and $\rho= 5.48$ g/cm³, we can estimate the ratio of decomposed H_2O_2 molecules per 1 NPs as $N(\text{H}_2\text{O}_2)/N(\text{NPs})=420:1$ or 0.3 molecules min⁻¹ per one NP.

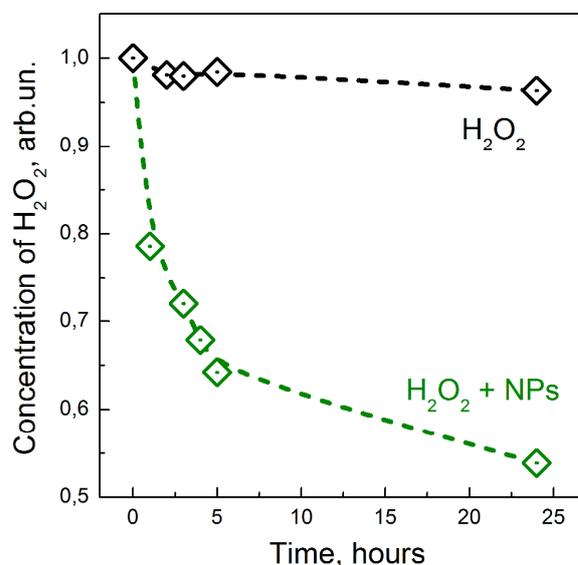


Fig. 3. Scavenging of H_2O_2 in water solutions containing synthesized orthovanadate nanocrystals.

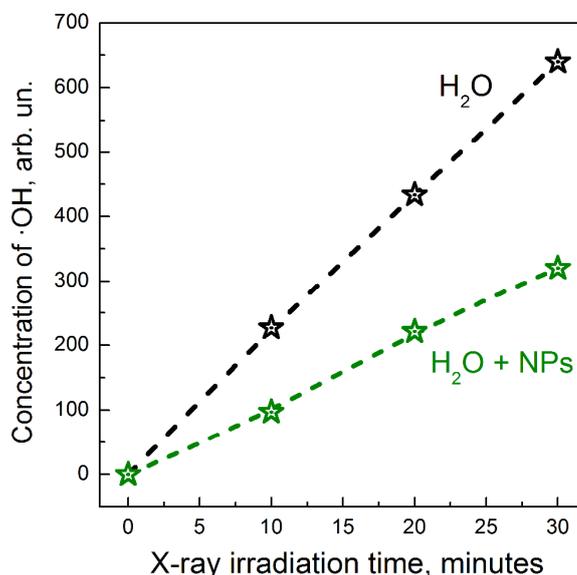


Fig. 4. Scavenging of $\cdot\text{OH}$ in water solutions containing synthesized orthovanadate nanocrystals at X-ray irradiation.

We also observe the antioxidant action of NPs against hydroxyl radicals, formed in the solution at X-ray irradiation as a main product of water radiolysis [18]. As it seen in Fig. 5, NPs decrease the concentration of $\cdot\text{OH}$ in the solution even after 30 min of X-ray irradiation. Thus, a large amount of V^{4+} and V^{3+} ions in the NPs crystal lattice allow NPs to be considered as potential antioxidant agents against the main ROS (H_2O_2 , $\text{O}_2^{\cdot-}$, $\cdot\text{OH}$) for the defense against ROS-mediated cellular damages.

Lipids of cell membranes, especially the polyunsaturated fatty acid residues of phospholipids are considered to be one of the main targets for ROS and their damage provokes oxidative stress [1,2,19]. ROS ($\text{O}_2^{\cdot-}$ and $\cdot\text{OH}$ commonly referred to as “free radicals”) can induce lipid peroxidation and disrupt the arrangement of membrane lipid bilayer. Lipid peroxidation is initiated when free radicals attack and abstract hydrogen from the methylene groups (CH_2) in a fatty acid. This results in formation of a carbon-centered lipid radical that can react with O_2 , generating a lipid peroxy radical (ROO^{\cdot}). The ROO^{\cdot} undergoes rearrangement via a cyclization reaction to form endoperoxides and other toxic

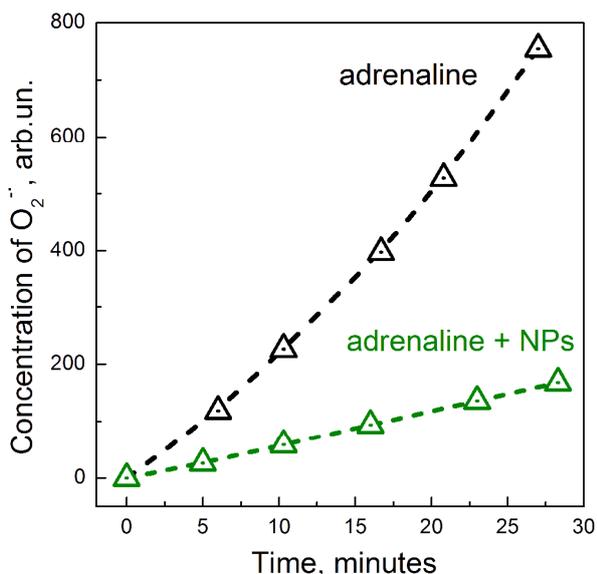


Fig. 5. Scavenging of $O_2^{\cdot-}$ in water solutions containing synthesized orthovanadate nanocrystals.

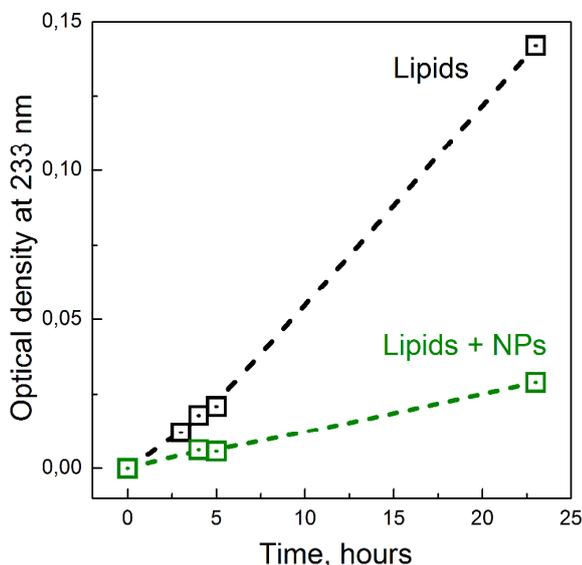


Fig. 6. Inhibition of heat-stimulated lipid oxidation in a water solution containing synthesized orthovanadate nanocrystals.

products responsible for the DNA and protein damage [19]. Moreover, $\cdot OH$ is reported to be responsible for the damage to biomolecules such as DNA and proteins [5]. At the first stage of lipid oxidation, the rearrangement of the double bonds of polyunsaturated fatty acids causes the formation of so-called conjugated dienes (CDs), and this process can be detected spectroscopically [17]. Such factors as heat, ionizing radiation and other environmental factors facilitate the lipid oxidation by activation of dissolved atmospheric oxygen. We analyze the effects of NPs on PC lipid autoxidation initiated by increased temperature ($65^\circ C$). Fig. 8 shows that in the PC lipid solution containing NPs, the concentration of CDs is much smaller. Even after 23 h of PC lipid suspension heating, in the solution with NPs CDs the concentration remains 75% smaller than that in the PC lipid solution without NPs. The observed effect could be also ascribed to $O_2^{\cdot-}$ and $\cdot OH$ radical scavenging by V^{3+} and V^{4+} at the first stage of lipid oxidation that inhibits their fast oxidation at high temperature.

IV. CONCLUSIONS

Taking all results together, we can conclude that in obtained gadolinium yttrium orthovanadate nanocrystals due to their structure, more than 60% of vanadium ions are presented in lower oxidation states (V^{4+} and V^{3+}). The coexistence of three oxidation states of vanadium ions is responsible for observed ROS scavenging activity of nanocrystals and, consequently, for their antioxidant properties. The obtained data can explain non-trivial anti-aging and radioprotective effects of rare-earth orthovanadate nanocrystals observed earlier *in vivo*.

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