Published in final edited form as:

Chem Commun (Camb). 2010 April 28; 46(16): 2736–2738. doi:10.1039/b922024k.

Nanoceria exhibit redox state-dependent catalase mimetic activity[†]

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Abstract

In this study we have found that cerium oxide nanoparticles exhibit catalase mimetic activity. Surprisingly, the catalase mimetic activity correlates with a reduced level of cerium in the +3 state, in contrast to the relationship between surface charge and superoxide scavenging properties.

Reactive oxygen species (ROS) are byproducts of normal oxygen metabolism that can cause oxidative damage to macromolecules in biological cells. Two dominant ROS, superoxide ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), are neutralized in cells by specific enzymes, primarily superoxide dismutase (SOD) and catalase, respectively. These enzymes thus act as antioxidants by protecting cells against the damaging effects of these two primary ROS. An intracellular imbalance between ROS and the enzymes that are involved in their detoxification can lead to a condition known as oxidative stress.¹ Oxidative stress has been implicated in several diseases and disorders, including Parkinson's and Alzheimer's disease,² cardiovascular disease³ and even tumor development.⁴ Additionally, the damage inflicted to cells by ROS and other reactive substances such as reactive nitrogen species (RNS) could be the root cause of aging and senescence, as per Harman's free radical theory of aging.⁵

Cerium oxide nanoparticles (nanoceria) have been shown to protect cells against ROS-induced damage.^{6–8} The small size of these nanoparticles gives them a high surface area to volume ratio, which is advantageous in that it provides a large surface for potential catalysis. Additionally, nanoceria are advantageous because of the ability of cerium to exist in either a +3 (reduced) or +4 (oxidized) state at the particle surface: Ce^{3+} are associated with oxygen vacancies. The crystal structure of cerium oxide shows most of the cerium atoms are present in the +4 state in the crystalline lattice. However, a reduction in particle size of nanoceria results in the formation of surface oxygen vacancies, which results in higher levels of cerium atoms in the +3 state,⁹ typically denoted as CeO_{2-x} .^{10,11} Previous studies from our lab have shown that this characteristic of nanoceria affects their catalytic efficiency as a scavenger of superoxide anions.^{12,13} Specifically, nanoceria were shown to exhibit SOD mimetic activity in a redox-state dependent manner, with the most efficient superoxide scavenging exhibited

[†]Electronic supplementary information (ESI) available: Nanoparticle synthesis, characterization and details on spectrophotometric analysis of peroxides. See DOI: 10.1039/b922024k

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by nanoparticles with higher levels of cerium in the +3 oxidation state at the particle surface.¹³

Based on this work, nanoceria have been studied as catalytic antioxidants by other groups in cell and animal model systems^{6,8,14–16} and each of these studies has shown that nanoceria can protect against oxidative stress that arises as a critical intermediate in each of these conditions. These models have included ROS involvement in neuronal excitotoxicity,⁶ cardiovascular disease,¹⁶ retinal degeneration,¹⁴ and radiation damage.⁸ In order to better understand how nanoceria provide protection in these environments, it is essential to establish any potential reactivity that these particles have with all physiologically relevant ROS, even RNS as well. As previously mentioned, nanoceria have been established as an SOD mimetic, by reacting with superoxide to form hydrogen peroxide. Other superoxide scavenging catalysts, especially manganese porphyrins, have also been shown to be catalase mimetics,¹⁷ *i.e.* to disproportionate hydrogen peroxide into molecular oxygen (O₂) and water. In this study we determined the reactivity of nanoceria with hydrogen peroxide on the basis of the similarity in the proposed mechanism (*i.e.* one electron transfer reactions catalyzed by redox cycling of the metal). Since the redox state of nanoceria has been shown to influence its catalytic activity towards superoxide, this variable was examined to assess any changes in reactivity with hydrogen peroxide.

In order to test any potential catalase mimetic activity of nanoceria, the Amplex Red assay to detect low levels of peroxide was first performed. This assay allowed us to detect any catalase mimetic activity at a physiologically relevant hydrogen peroxide concentration (2 μ M). The results from the Amplex Red assay showed that nanoceria preparations B, which has higher levels of cerium in the +4 state, exhibited significant catalase mimetic activity, while preparation A, with a higher level of surface cerium atoms in the +3 state, did not (Fig. 1). This initial result strongly suggested that the breakdown of hydrogen peroxide by nanoceria was dependent on their +3/+4 redox-state ratios, and that the reactivity with peroxide did not parallel the reactivity with superoxide. The measured enzymatic rate of nanoceria preparation B was 9.08×10^{-4} nmol min⁻¹.

Our results thus show that nanoceria act as catalase mimetics in a redox-state dependent manner, since only nanoceria preparations with fewer surface cerium atoms in the +3 state displayed any substantial catalase mimetic activity. These findings are of particular interest because nanoceria with lower +3/+4 ratios were found to be less efficient in their SOD mimetic activity, and thus the catalysis appears to be inversely related with respect to the steady state cerium oxidation state of the nanoparticle.

In order to corroborate the data obtained from the Amplex Red Assay, we followed changes in peroxide levels using UV-Vis spectrophotometry. This allowed us to follow hydrogen peroxide levels in a real-time kinetic manner. However, a higher concentration (10 mM) of H₂O₂ was needed in the assay to be able to monitor H₂O₂ levels given its extinction coefficient. Our results from this experiment supported our previous findings, in that the catalase mimetic activity of nanoceria was dependent on the redox-state ratios of the nanoceria preparations. Nanoceria preparation B, with a higher level of cerium in the +4 oxidation state, led to more rapid decreases in the concentration of hydrogen peroxide at a rate of 2.71 nmol min⁻¹ (Fig. 2). In contrast nanoceria preparation A, with a higher concentration of cerium in the +3 state did not exhibit any detectable catalase mimetic activity under these conditions at these concentrations. We observed concentration-dependent decrease in peroxide in the presence of varying levels of preparation B (Table 1).

Finally, to confirm that nanoceria were causing the breakdown of hydrogen peroxide to molecular oxygen (O₂), we measured changes in dissolved oxygen production under similar

reaction conditions. However, a higher hydrogen peroxide concentration (100 mM) had to be used in order to be able to detect the production of dissolved oxygen. These results again agreed with our previous findings about the role of the redox state of the cerium atom in its catalase mimetic activity (Fig. 3). Nanoceria with higher +3 levels were not as efficient to catalyze the formation of oxygen in the presence of peroxide, while cerium oxide nanoparticles with higher levels of surface atoms in the +4 state were effective. Two other nanoceria preparations (refer ESI[†]), ceria nanorods and ceria nanocubes, were also tested and were found to also display substantial catalase mimetic activity. The synthesis was carried out using established hydrothermal techniques and has been listed in the ESI. [†] 10

Table 2 shows the relationship between catalase mimetic activity (as measured by oxygen production as described in Fig. 3) and the surface chemistry of nanoceria. The level of surface cerium atoms in the +3 oxidation state inversely correlates with the ability of these nanomaterial catalysts to exhibit catalase mimetic activity.

Since each preparation of cerium oxide nanoparticles may also have altered surface chemistry that is poorly understood, we then decided to attempt to alter the redox state of an existing preparation of nanoceria. Only exposure to hydrogen peroxide has been shown to alter the level of cerium atoms in the +3 state, and this is indeed the substrate in the described catalytic reaction.¹² Fortunately we have recently found that incubation of nanoceria with phosphate solutions (such as phosphate buffered saline) can over time alter the redox state of cerium at the particle surface. A clear shift from +3 to +4 state is confirmed by UV-visible spectrophotometry in a prepared sample (ESI[†] Fig. S3A). This shift also led to an improved catalase mimetic activity for this sample of cerium oxide nanoparticles, showing direct evidence that by altering the level of cerium in the +3 state, the catalytic activity with hydrogen peroxide is improved (ESI[†] Fig. S3B). It should be noted that the chemical properties of cerium oxide at the nanoscale is still a rapidly progressing area, and our ongoing studies are focused on understanding the mechanism of how peroxide can interact with cerium atoms at the particle surface.

In summary, this study establishes the catalase mimetic capabilities of nanoceria and affirms that this catalytic reaction is not equivalent for all nanoceria preparations. Our findings suggest that one of the mechanisms by which nanoceria protect cells from oxidative stress may be due to their ability to act as catalase mimetics, in addition to exhibiting SOD mimetic activity. Previous studies that have shown antioxidant activity of nanoceria should be reevaluated in terms of the mechanism of action of these materials *in vivo*.

Supplementary Material

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Acknowledgments

These studies were supported by a grant from NIH (1R01AG031529-01) and grants from NSF (0708172 CBET, 0804733 CBET) to W. T. S. and S. S.

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Fig. 1.

Nanoceria exhibit catalase mimetic activity at physiologically relevant hydrogen peroxide concentrations in a redox-state dependent manner. Hydrogen peroxide levels were quantified using the Amplex Red assay. Ceria preparation A (open triangle, 100 μM , filled circles, 300 μM), with a higher +3/+4 redox state ratio, exhibits no catalase mimetic activity at this concentration of hydrogen peroxide. Preparation B (filled squares, 100 μM and open circles, 300 μM), with relatively low +3/+4 redox-state ratios, exhibits substantial catalase mimetic activity.

Fig. 2.

Nanoceria with higher levels of cerium in the +3 oxidative state are inefficient catalase mimetics. Any baseline changes that occur in a sample with peroxide alone (negative alone) have been subtracted from the raw data, hence normalized absorbance is plotted. Hydrogen peroxide was followed by changes in absorbance of the reaction monitoring absorbance at 240 nm. The slight changes in absorbance peroxide control (no nanoceria) filled circles; nanoceria preparation A with peroxide, open circles; nanoceria preparation B, filled inverted triangles. The mean of at least three independent experiments is plotted with the standard deviation of the mean shown as error.

Fig. 3.

Nanoceria catalyze the conversion of hydrogen peroxide to oxygen. The level of dissolved oxygen was monitored using a standard Clark electrode in air-saturated buffer in the presence of 0.1 M hydrogen peroxide, DTPA and cerium oxide nanoparticles. Without the addition of nanoparticles no detectable changes in dissolved oxygen were observed. A dynamic Weibull peak fit curve was used to smooth the data from the raw probe data since the sensitivity of the instrument is only up to 0.1%, using SigmaPlot 10.

Table 1Changes in peroxide levels in the presence of nanoceria^a

[H ₂ O ₂]/mM	10 μ M CeO ₂	50 μ M CeO ₂	100 μ M CeO ₂
0.5	0.35	1.09	1.61
1.0	0.32	1.16	2.05
5.0	0.85	1.94	2.57
10.0	0.93	2.27	2.71

^aRates are nmol min⁻¹ and were calculated by determining the rate of changes in absorbance per unit time, based on the molar extinction coefficient of hydrogen peroxide at 240 nm (43.6 M⁻¹ cm⁻¹).

Table 2Correlation between cerium redox state in nanomaterials and catalase mimetic activity^a

Preparation	Dissolved O ₂ levels (%)	+3/+4 ratio
A	0.16 ± 0.05	28.83
B	0.85 ± 0.13	6.69
Nanorods	0.35 ± 0.06	7.31
Nanocubes	0.20 ± 0.08	8.55

^a Changes in the dissolved oxygen levels were measured using the Clark oxygen electrode as discussed in experiment methods (ESI[†]). The level of cerium atoms in the +3 oxidation state was determined by X-ray photoelectron spectroscopy from the analysis of Ce3d orbital spectrum (ESI[†]).