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The effect of visible light on gold nanoparticles and some bioeffects on environmental fungi

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ABSTRACT

The oxidative stress induced by light exposed gold nanoparticles in some microorganism cells was investigated. Gold nanoparticles are currently used in biomedical and pharmaceutical research. For this study citrate-gold nanoparticles were synthesized in alkaline conditions at constant temperature of 85 °C under magnetic stirring. Equal volumes of such prepared colloidal solution, were exposed to visible light at different wavelengths for 90 min at room temperature. The spectra in the visible and ultraviolet range have revealed an increase in the intensity of the absorption band for gold nanoparticles exposed to light, due to the effect of surface plasmon resonance. Versatility of gold nanoparticles photocatalytic action was shown by means of manipulating wavelengths of incident light, which evidenced differences in the bioeffects induced in cellulolytic fungi – known for their environmental role but also for other applications such as in cosmetics industry. The comparative analysis of fungal response to gold nanoparticle stressors has revealed different enzyme activity and lipid peroxidation when fungi were supplied with gold nanoparticles exposed to different wavelength lights. The activity of catalase and superoxide dismutase were remarkably increased for green light exposure of gold nanoparticles suggesting fungi adaption to increased oxidative stress induced by irradiated particles; increased level of lipid peroxidation was showed by high concentration of malondialdehyde for white light exposed gold particles since antioxidant enzymes were less active.

1. Introduction

In this paper we present an experimental study on the oxidative stress induced by gold nanoparticles (AuNPs) in the cells of environmental fungi *Phanerochaete chysosporium* having in mind both the injuring effects of nanoparticulated metal released in the waters and soils and the possible monitoring of cultivated cellulolytic fungi to yield useful enzymes when supplied with AuNPs.

P. chrysosporium is known mainly for its cellulolytic properties demonstrated in natural environment; based on such features, biotechnological applications have been developed for bioremediation of lead-contaminated soil and for degradation of various xenobiotic compounds (Volkan et al., 2011); also lignin peroxidase yielded by this microorganism could be used in cosmetics against melanin excess in skin (Woo et al., 2004).

It is the enzymatic activity that makes these microorganisms equally interesting both for environmental studies and for pharmaceutical research. In some scientific reports the oxidative capability of *P. chrysosporium* was demonstrated for various recalcitrant compounds (Wesenberg et al., 2003) structurally similar to lignin (synthetic dyes, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pharmaceutical products and endocrine disrupting reagents); for instance anti-inflammatory drugs (Rodarte-Morales et al., 2012) released in waste waters from

Abbreviations: AuNPs, gold nanoparticles; ROS, reactive oxygen species; SOD, superoxidedismutase; CAT, catalase; MDA, malondialdehyde; LSPR, localized surface plasmon resonance; TEM, transmission electron microscopy; DLS, dynamic light scattering; DF, dark-field optical microscopy; P1C, control; GF, green light filter; BF, blue light filter; YF, yellow light filter; WL, white light; SPR, surface plasmon resonance; UV–vis, ultraviolet–visible.

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pharmaceutical industry were degraded by *P. chrysosporium* as well as toxic residues of tetracycline and oxytetracycline (Wen et al., 2009).

In this context, during last decades, increased scientific interest in the limitation of reactive chemical species formation by stimulating defense mechanisms of living cells could be seen (Bai et al., 2003). Energy-rich unstable chemical species—mostly reactive oxygen species (ROS) normally appear during complex metabolic processes in almost all cells and tissues. Consequently ROS trigger chemical reactions with various cell molecular components, thus generating the so called oxidative stress, especially when natural antioxidant defense mechanisms in the cell are overhelmed. Cell defense system can be stimulated to intensify the biosynthesis of enzymes that act as antioxidants and protect cellular components against oxidation caused by reactive oxygen species (ROS) (Woo et al., 2004). ROS level can be changed under the action of (photo)catalysts such as metal nanoparticles supplied into the cells. Antioxidant defense system involves enzymatic systems such as superoxidedismutase (SOD), glutathione peroxidase (GPO), catalase (CAT), as well as nonenzymatic factors.

Evaluation of oxidative stress may be achieved by assaying, for instance, the concentration of malondialdehyde (MDA) as an indicator of peroxidation of polyunsaturated fatty acids in cell membranes. Several reports have shown that ROS, when below lethal threshold, can have also some positive effects; for example ROS levels may influence intracellular signaling pathways in neural system (Tsatmali et al., 2006) as well as in plant cells (Dröge, 2002) by stimulating not only the cellular defense systems but also other metabolic pathways; or, reactive oxygen species could be able of stimulating the differentiation of pathogenic eukaryotes (Aguirre et al., 2005); some studies have shown that ROS can influence differentiation of cardiac stem cells, and of renal cells—mediated by angiotensin (Puceat, 2005).

Photocatalytic processes are known to play an important role not only in materials science, but also in energy storage, and environmental remediation (Wittstock et al., 2010). Metal nanoparticle increased catalytic performance, as in the case of gold nanoparticles (AuNPs), may be achieved based on the energy absorbed during light exposure being evidenced by the effect of localized surface plasmon resonance (LSPR) on the surface of AuNPs when exposed to visible light (Campbell et al., 2002). LSPR effect is given by collective electron charge oscillations in metallic nanoparticles that are excited by light absorption, resulting in interband transition of metal electrons (Linic et al., 2011). This procedure was used for the oxidation of organic contaminants and aromatic alcohols (Chen et al., 2008). Under visible light irradiation, the 6sp electrons of AuNPs undergo an intraband excitation to higher energy level and left positive charges in the ground level, which can capture electrons from reactant molecules to oxidize them (Zhu et al., 2009). The aim of photocatalysis is to utilize photon energy initiating electron transfer and resulting in chemical reactions. If nanoparticles associate to reactive compounds, their energy can be used to initiate chemical transformations.

Photocatalytic performance of the nanoparticles can be adjusted by changing their size, shape and also the properties of their environment. These adjustments can generate LSPR peak position changes. Recent reports have shown that the photocatalytic reduction can be optimized by manipulating the wavelength of the incident radiation (Zhu et al., 2010); to control the cell antioxidant response, light exposed gold nanoparticles can be used as stressors with modulation of light wavelength (Silva et al., 2011).

The study presented below examines how the fungi *P. chrysosporium* protects themselves against the effects of reactive chemical species induced by nanoparticulate stressors irradiated with different wavelength light beams.

2. Materials and methods

2.1. Reagents

The chemicals used in the AuNPs synthesis were hydrogen tetrachloroaurate (III) trihydrate ($HAuCl_4 \cdot 3H_2O$), sodium hydroxide (NaOH) and sodium citrate dihydrate ($C_6H_9Na_3O_9$), all purchased from Sigma Aldrich. The solutions were prepared using Milli-Q deionized water.

2.2. Biological material

The cellulolytic microorganism *P. chrysosporium*, was acquired from the Institute Sciéntifique de Santé Publique, Belgium (HEM no. 5772).

2.3. Investigation methods

2.3.1. Particle size distribution

2.3.1.1. Particle hydrodynamic diameter in colloidal suspension. To find the hydrodynamic diameter of gold nanoparticles in colloidal suspension a Malvern Zetasizer Nano ZS, model Zen-3500, working based on dynamic light scattering (DLS) was used at room temperature.

2.3.1.2. Particle size by TEM measurement. Transmission electron microscopy (TEM) investigation was carried out with Philips CM100 that enabled morphological and micrographic analysis of citrate-AuNPs samples deposited on carbon covered grid. Size analysis of TEM micrographs on standard scale, has been done using the specialized research software NIS Elements (NIS-BR).

2.3.1.3. Particle plasmon imaging. The recording of AuNP images in Dark-field optical microscopy (DF) was performed using a Nikon Ti-.Eclipse microscope.

2.3.2. Spectral investigation

The spectral behavior in ultraviolet and visible range of irradiated suspension was analyzed with Shimadzu UV–vis spectrophotometer using 1 cm quartz cuvette.

2.4. Gold nanoparticles synthesis

5 ml of $HAuCl_4 \cdot 3H_2O$ (1 mM) and 1.75 ml NaOH (20 mM) were mixed in a suitable glass flask that was filled with distilled and deionized water up to 50 ml. The flask was immersed in a water bath preheated to 110 °C using a thermostatic plate. After about 30 min, the solution temperature reached 85 °C, and then, 2.5 ml sodium citrate (50 mg/ml) was added. The flask was let on the thermostatic plate under magnetic stirring, for about 15 more minutes at the same temperature until the solution color turns pink. At this moment the flask was removed from the source of heat and immersed in cold water to 4 °C to stop the process of nucleation.

2.5. AuNPs irradiation

Equal volumes of 10 ml from the stock solution were prepared for irradiation with light (Table 1) emitted by a 50 W halogen lamp at 24.5 cm height. P1 nonirradiated sample contains non-irradiated AuNP colloidal suspension. Each sample was irradiated in series of 5 min duration with breaks of 5 min, totalizing 90 min light