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Antioxidant and cytotoxic effect of biologically synthesized selenium nanoparticles in comparison to selenium dioxide

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ABSTRACT

The present study was designed to evaluate antioxidant and cytotoxic effect of selenium nanoparticles (Se NPs) biosynthesized by a newly isolated marine bacterial strain *Bacillus* sp. MSh-1. An organic–aqueous partitioning system was applied for purification of the biogenic Se NPs and the purified Se NPs were then investigated for antioxidant activity using DPPH scavenging activity and reducing power assay. Cytotoxic effect of the biogenic Se NPs and selenium dioxide (SeO_2) on MCF-7 cell line was assesed by MTT assay. Tranmission electron micrograph (TEM) of the purified Se NPs showed individual and spherical nanostructure in size range of about 80–220 nm. The obtained results showed that, at the same concentration of 200 μg/mL, Se NPs and SeO_2 represented scavenging activity of $23.1 \pm 3.4\%$ and $13.2 \pm 3.1\%$, respectively. However, the data obtained from reducing power assay revealed higher electron-donating activity of SeO_2 compared to Se NPs. Higher IC_{50} of the Se NPs (41.5 ± 0.9 μg/mL) compared to SeO_2 (6.7 ± 0.8 μg/mL) confirmed lower cytotoxicity of the biogenic Se NPs on MCF-7 cell line.

Introduction

Oxidative stress and uncontrolled production of reactive oxygen species (ROS) have been identified as one of the most important reasons in pathological progression of many diseases and several kinds of cancers [1,2]. Different mechanisms such as enzymatic conversion of free radicals and detoxification using antioxidants have been developed by living cells to defend against dangerous effect of the mentioned radical intermediates [2,3]. So, introducing novel, efficient and cost effective antioxidants with lower toxicity has been the aim of many investigations [4,5].

Selenium (Se) is an essential dietary trace element, the antioxidant activity of which has been supported by a large number of clinical and epidemiological studies [2,6]. This micronutrient metalloid is the main component of selenoenzymes which are found to protect animal cells from oxidative damage [6,7]. Nutritional deficiency of Se is the main reason of Keshan disease, age-related degenerative diseases and muscular dystrophy in humans [7,8]. Stimulation of immune responses [8] and reduction of overall cancer mortality [2,9] are among other advantages of Se intake determined by recent studies. However, dose and chemical form of selenium derivatives play an important role in both their bioavailability and biological activities [1,6]. Excellent biological properties of selenium nanoparticles (Se NPs) together with their lower toxicity have introduced them as a good candidate for replacing other forms of selenium in nutritional supplements [8,9].

There are several reports on the ability of bacterial strains like *Bacillus megaterium* [10], *Pseudomonas alcaliphila* [11] and *Enterobacter cloacae* SLD1a-1 [12] for producing Se NPs. An efficient Se NPs producer bacterial strain was recently isolated from the Caspian Sea and identified as *Bacillus* sp. MSh-1 [13]. In the present study, the biologically synthesized Se NPs was purified from the whole-cell lysate of *Bacillus* sp. MSh-1 and the antioxidant and cytotoxic activities of the purified Se NPs were evaluated compared with selenium dioxide. To the best of our knowledge and according to the literature review, limited works have been conducted to assay both antioxidant and cytotoxic properties of the biogenic Se NPs.

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Materials and methods

Chemicals

2,2-Diphenyl-1-picrylhydrazyl (DPPH) and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Selenium dioxide (SeO_2) and butylated hydroxytoluene (BHT) were provided by Merck Chemicals (Darmstadt, Germany). Fetal bovine serum (FBS), Dulbecco's modified Eagle medium (DMEM) and antibiotics were supplied by Gibco (Life Sciences Inc., USA). All other chemicals and solvents were of analytical grade.

Bacterial strain, biosynthesis and purification of the Se NPs

The bacterial strain used for biosynthesis of Se NPs in the present study was previously isolated from the Caspian Sea and identified as *Bacillus* sp. MSh-1 based on 16S rDNA gene analysis [13]. Se NPs were prepared according to a recent method described by Shakibaie et al. [13]. Briefly, sterile nutrient broth (NB) medium containing selenium dioxide (SeO_2, final concentration 1.26 mM) was inoculated with 1 mL of the fresh inoculums (OD_{600}, 0.1) of *Bacillus* sp. MSh-1 and incubated in a shaker incubator (150 rpm) at 30 °C for 14 h. The bacterial cells were then harvested by centrifugation at 4000 × *g* for 10 min. After washing the obtained pellets with sterile solution of NaCl (0.9%) for three times, the bacterial cells were disrupted by grinding the frozen cells in liquid nitrogen using a mortar and pestle. The resulting slurry was then ultrasonicated at 100 W for 5 min and washed three times by sequential centrifugation (14,000 × *g*, 5 min) with a 1.5 M Tris–HCl buffer (pH 8.3) containing 1% SDS and deionized water, respectively. Subsequently, Se NPs were extracted and purified by organic–aqueous partitioning system (*n*-octanol–water), as previously described [13]. Micrographs of the prepared biogenic Se NPs were obtained using a TEM apparatus (Zeiss 902A) operated at accelerating voltage of 80 kV. The related size distribution pattern of Se NPs was plotted by manual counting of 400 individual particles from different TEM images.

DPPH scavenging activity of the purified Se NPs

Purple DPPH radicals changes into a yellow stable compound in presence of an antioxidant and the extent of the reaction depends on hydrogen donating ability of the antioxidant [14]. DPPH scavenging activity of the biogenic Se NPs was determined according to the method described by Turlo et al. [4] with some modifications. One mL of Se NPs or SeO_2 solution (20–200 μg/mL) was mixed with 1 mL of the freshly prepared DPPH solution in methanol (0.15 mM). After addition of methanol (3 mL), the mixture was incubated in dark at room temperature for 30 min and absorbance of the mixture was then recorded at 517 nm using a UV–vis spectrophotometer (UVD-2950; Labomed). The negative control was designed by replacing Se NPs or SeO_2 stocks with deionized water. Scavenging percentage of DPPH was calculated as follows:

$$\text{DPPH radical scavenging ability } (\%) = \left[1 - \frac{(A_a - A_b)}{A_c}\right] \times 100$$

where A_a is absorbance of the sample mixed with DPPH solution, A_b is absorbance of the sample without DPPH solution and A_c is absorbance of the control solution. Required concentration of the test samples for inhibiting 50% of DPPH (IC_{50}) was calculated by linear regression. The same experiment was performed for BHT as positive control at similar concentration. All the experiments were carried out in triplicate and mean of the obtained results was reported.

Reducing power assay

Reducing power of Se NPs and SeO_2 was determined by modified protocol of Oyaizu et al. [4,15]. This assay is based on reduction of Fe^{3+} to Fe^{2+} in the presence of antioxidants. Briefly, 1 mL from different concentrations of Se NPs or SeO_2 (20–200 μg/mL) was mixed with 0.5 mL of sodium phosphate buffer (0.2 M, pH 6.6) and 0.5 mL of potassium ferricyanide (30 mM). The mixture was then incubated at 50 °C and 100 rpm for 20 min. Thereafter, 2 mL of trichloroacetic acid (TCA, 0.6 M) was added to the above mixture followed by centrifugation at 3000 rpm for 10 min. The obtained supernatant (0.5 mL) was mixed with deionized water (0.5 mL) and 0.1 mL of the $FeCl_3$ solution (6 mM) and the absorbance was measured at 700 nm. The negative control was designed by incubating the mentioned reaction mixture in absence of Se. The same experiment was also repeated for BHT as a reference compound at similar concentration. These procedures were replicated three times on different days, and mean of the absorbencies was used to draw a suitable curve.

Cell culture and cytotoxicity assay

MCF-7 cell lines were obtained from the National Cell Bank of Iran, Pasteur Institute of Iran (Tehran, Iran). The cells were maintained in DMEM medium supplemented with FBS (10%, v/v) and antibiotics [penicillin (100 units/mL) and streptomycin (100 μg/mL)] at 37 °C in a CO_2 incubator (5% CO_2 and 95% relative humidity). In order to evaluate cytotoxic effect of the Se NPs and SeO_2, MCF-7 cells were harvested in the exponential phase of growth, seeded into 96-well tissue culture plates (15,000 per well) and allowed to adhere for 24 h. Thereafter, Se NPs and SeO_2 were added to the desired wells to reach final concentration of 0–200 μg/mL. After 24 h of incubation, 20 μL of DMEM medium containing MTT (5 mg/mL) was added to each well and incubated for 4 h. Consequently, the medium was replaced with 100 μL of DMSO, and optical densities were determined at 570 nm. MTT assay was performed in three replicates for each experiment.

Statistical analysis

Each value was expressed as mean ± SD. SPSS software 15 for windows (SPSS Inc., Chicago) was used for statistical analysis. Differences between the groups were determined using one-way analysis of variance (ANOVA) and *p*-values less than 0.05 were considered significant.

Results

Biosynthesis of Se NPs

The obtained results confirmed that bacterial strain of *Bacillus* sp. MSh-1 effectively reduced Se^{4+} to elemental Se which was evident from the color change of cultivation medium to orange-red due to generation of Se(0) during the exponential growth phase (the data were not shown). The biologically synthesized Se NPs was successfully purified using *n*-octanol–water partitioning system. According to Fig. 1 which shows TEM image of the purified nanostructures, Se NPs represented spherical shape and most of them were in the size range of 80–220 nm. TEM image clearly illustrated individual Se NPs with a small amount of aggregation. Size distribution pattern (Fig. 2), measured from manual counting of 400 individual particles from different TEM images, revealed that the most frequent particles were in size range of 125–150 nm.