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Selenium nanoparticles synthesized in aqueous extract of *Allium sativum* perturbs the structural integrity of Calf thymus DNA through intercalation and groove binding

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ABSTRACT

Biomedical application of selenium nanoparticles (SeNPs) demands the eco-friendly composite for synthesis of SeNPs. The present study reports an aqueous extract of *Allium sativum* (*AqEAS*) plug-up the current need. Modern spectroscopic, microscopic and gravimetric techniques were employed to characterize the synthesized nanoparticles. Characterization studies revealed the formation of crystalline spherical shaped SeNPs. FTIR spectrum brings out the presence of different functional groups in *AqEAS*, which influence the SeNPs formation and stabilization. Furthermore the different aspects of the interaction between SeNPs and CT-DNA were scrutinized by various spectroscopic and cyclic voltametric studies. The results reveals the intercalation and groove binding mode of interaction of SeNPs with stacked base pair of CT-DNA. The Stern–Volmer quenching constant (K_{SV}) were found to be 7.02×10^6 M^{-1} (ethidium bromide), 4.22×10^6 M^{-1} (acridine orange) and 7.6×10^6 M^{-1} (Hoechst) indicating strong binding of SeNPs with CT–DNA. The SeNPs - CT-DNA interactions were directly visualized by atomic force microscopy. The present study unveils the cost effective, innocuous, highly stable SeNPs intricate mechanism of DNA interaction, which will be a milestone in DNA targeted chemotherapy.

1. Introduction

The unique physicochemical properties of nanoparticles deliver them as a promising competitor in the field of biomedicine [1]. Metal nanoparticles have obtained great scientific interest owing to their peculiar characteristics. Among the various metal nanoparticles, studies an selenium nanoparticles (SeNPs) has been a remarkable area of research due to their bioavailability and less toxicity [2]. Selenium (Se) is an essential micro-nutrient and vital dietary trace element which not only exerts its role as a key component of several major metabolic pathways but also augments immune function, lowering the generation of free radicals, maximizing the thyroid function and protecting against heart diseases. Both inorganic and organic forms of selenium have prevalent role in the physiological and biomedical applications [3]. On the other hand, toxicity of selenium is one of the remaining intricacies in the biological applications. Nanosize selenium can overwhelm the low therapeutic index of organic and inorganic forms of selenocompounds due its unique physiochemical characteristics. SeNPs have been found as an excellent compound of antioxidant, antiviral, antimicrobial, antifungal and anticancer activities [4,5]. Synthesis of bio-compatible SeNPs is challenging, because chemical-mediated synthesis of nanoparticles has its own demerits such as high energy consumption and expensiveness. On the other hand green synthesis has become the focus of extensive research owing to their various advantages including cost effectiveness, trouble-free harvesting, etc. [6].

Green synthesis exploits bio-reductants naturally present in plants, bacteria, fungi and algae [7]. Several drawbacks associated with microorganism mediated biosynthesis of nanoparticles, such as pathogenecity of microbes, time requirement and post harvesting, have made the thrust behind plant extract-mediated nanoparticle synthesis [8]. Synthesis of SeNPs from different plant extracts have been reported [9,10]. The efficiency of green synthesis depends on numerous bioreductants present in plant, from this green facet in the present study, we intended to study the reducing and stabilizing property of pharmacologically valued plant *Allium sativum (AS)*, a member of amaryllidaceae family, cultivated throughout the world. *AS* is rich with an organosulfur compound allicin, which has been documented to contribute potent biological activity of *AS*, apart from this allin, allyl sulfide, ajoene, 1, 2, vinyldithin are also present in *AS*. Its biological effects range

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from antidiabetic, anti-inflammatory, antifungal, antioxidant, immunomodulatory and cardioprotective [11,12,13]. In addition garlic extract plays an essential role in the suppression of proliferation in cancer cell [14,15].

Molecular interactions between DNA and nanoparticles have drawn attention of researchers, because DNA has emerged as a promising target in chemotherapy. Maintenance of DNA integrity is essential for relevant cellular function and proliferation. The interaction of nanoparticle can induce DNA damage and induce cell cycle arrest which in turn adversely affects other functions of DNA such as replication, transcription and finally leading to cell death [16,17]. However, direct interaction of SeNPs with DNA is not an impossible mode of action, because recent studies have shown the direct interaction of nanoparticle with DNA [18,19]. Recent study has reported the synthesis of SeNPs using *Trigonella foenum graecum* extract and its interaction with herring sperm DNA [20].

In the present study, we deliberate to study the biosynthesis of SeNPs using the reducing power of *AS* and the direct interaction of SeNPs with Calf thymus DNA (CT-DNA), such an interaction would be helpful to develop SeNPs as a sole therapeutic molecule for targeted chemotherapy.

2. Materials and methods

2.1. Materials

Selenious acid was purchased from Merk Milli Pore, USA. Sodium alginate, ascorbic acid and ethanol were purchased from Himedia, India. CT-DNA was purchased from Sigma Aldrich, India. *Allium sativum*, commonly known as garlic was purchased from local market between July 2015 and December 2015, Pondicherry, India, authenticated by Botanist Prof. B. Kannabiran (Retired), Department of Biochemistry and Molecular Biology, Pondicherry University, India. For all preparations ultra pure Milli-Q water was used.

2.2. Preparation of garlic extract

Fresh garlic bulbs were rinsed with running tap water and then washed with distilled water. Aqueous extract was prepared in Milli-Q water. The garlic bulbs were peeled and their cloves were isolated and grounded in mortar and pestle by the addition of Milli-Q water. The extract was filtered through muslin cloth followed by Whatman No. 1 filter paper. Finally, the extract was centrifuged at 10,000 rpm for 5 min. The supernatant was collected and used for further studies.

2.3. Synthesis of SeNPs

For the synthesis of SeNPs, 2 ml of garlic extract (2%) was mixed with 8 ml of 30 mM selenious acid, to this mixture 200 μl of 40 mM ascorbic acid was added. Ascorbic acid was used as an initiator of reduction process. Further 0.2% sodium alginate, 200 μl of 40 mM ascorbic acid and selenious acid mixture was used as standard positive control for SeNPs synthesis. The mixture of 2% extract and 200 μl of 40 mM ascorbic acid were used as negative control. All preparations were carried out at the room temperature. After 48 h of incubation, the preparation was centrifuged at 15,000 rpm for 15 min. The resulting pellet was washed with Milli-Q water followed by ethanol three times, and the washed pellet was dried overnight. The powder form of SeNPs was used for subsequent studies.

2.4. Characterizations of the synthesized SeNPs

The bioreduction of selenious acid into SeNPs in solution was monitored by sampling an aliquot (1 ml) of the mixture at 12 and 24 h, the UV–visible spectrum (Shimazdu 1700, UK) was recorded in the wavelength ranging between 200–800 nm. The size distribution and average molecular weight of synthesized SeNPs were measured by using DLS instrument (Malvern Particle Size Analyzer; MS 2000). The phases (crystalline or amorphous) of SeNPs were determined by X–ray diffraction study. The XRD pattern of powdered SeNPs was recorded using Rigaku ultima IV X-ray diffractometer with CuKα radiation ($\lambda = 1.54059$ A°) and data were collected within the scanning range of diffraction angle at $10° \leq 2\theta \leq 80°$. The crystalline qualities and lattice dynamics of SeNPs was investigated by Raman spectrometer (Renishaw, NRS–3100, UK) with a wavelength of 785 nm and argon laser was used as an excitation source with an incident laser power 30 mW. The vibrational modes of functional group of the compounds were analyzed by Fourier transform infrared spectroscope (FTIR), Thermo Nicolet model 6700, USA spectrum instrument in the transmittance mode, 4000–500 cm^{-1}, at a resolution of 4 cm^{-1} was used. The sample was prepared by mixing and fine grinding of powdered SeNPs with potassium bromide (KBr) in the ratio of 1:10, the samples were made in FTIR discs. The obtained peaks were plotted as % of transmittance in X–axis and wave number (cm^{-1}) in Y–axis. The surface morphology and size of SeNPs were analyzed by scanning electron microscopy (Hitachi-S-3400 N, Japan) with resolution of 500 nm operated at an accelerating voltage of 15.0 Kv HV mode and detectors containing secondary electron. The elemental composition of the SeNPs was obtained from coupled EDX spectra. The prepared SeNPs were dispersed in absolute ethanol and a drop of this suspension was placed on a sample loading grid with carbon sputter coating in ultra vacuum. After complete drying in the ultra vacuum, the images were captured followed by elemental analysis. The three dimensional structure of SeNPs was analyzed by atomic force microscopy (AFM). A clean glass cover slip was cut into 1×1 cm dimensions, cleaned in a piranha solution (hydrogen peroxide and sulphuric aicd; 30:70 ratio) and then washed thoroughly with Milli-Q water and kept in oven at 80 °C for 2 h. About 1 mg/ml concentration of SeNPs were prepared in ethanol, a drop of SeNPs suspension was placed on clean cover slip and kept in vacuum dessicator overnight, a thin film was formed over the cover slip. The dried nanoparticle suspension was scanned by AFM (Bruker MM8) in room temperature at scan rate of 0.996 Hz in air tapping mode attached with a nanoscope controller (manufactured in Santa Barbara,USA). Images were recorded in tapping mode in air with nanoprobe cantilever made of silicon nitride. Decomposition of SeNPs and impurities present in green synthesized SeNPs was analyzed through DT–TG analysis (SDT Q600 V20.9 Build 20, Australia). 10 mg of SeNPs powder was analyzed using a DT–TGA analyzer in the presence of nitrogen atmosphere in the temperature range from 49 °C to 250 °C, with an increasing temperature of 10 °C min^{-1}.

2.5. Studies of the CT-DNA- SeNPs interaction

To study the CT-DNA–SeNPs interactions, stock solution of CT-DNA preparation and CT-DNA purity was performed as mentioned in Subastri et al., [21].

2.6. Interaction studies

The alteration of the absorption spectrum of DNA with stepwise addition of SeNPs was studied by UV - visible spectrophotometer. Study was carried out at 298 K using Shimadzu spectrophotometer with a 1 cm path length rectangular quartz cuvette, in the wavelength range of 200–800 nm. Steady state and competitive displacement fluorescence assays were used to investigate the type of quenching and mode of interaction of SeNPs with CT–DNA. Fluorescence measurement was carried in Flouorolog FL3–11 spectrofluorometer. In steady state fluorescence, the spectra of SeNPs were recorded in the range of 285 nm to 525 nm upon excitation at 274 nm. The change in fluorescence intensity was observed by adding the fixed concentration of SeNPs (9 μM) with different concentrations of CT-DNA from 5 to 100 μM at temperature 298 K. After addition of each concentration, the final reaction mixture was made to 3 mL by adding 10 mM Tris–