Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 141 (2015) 176–184

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Interaction studies between biosynthesized silver nanoparticle with calf thymus DNA and cytotoxicity of silver nanoparticles

Swarup Roy *, Ratan Sadhukhan, Utpal Ghosh, Tapan Kumar Das

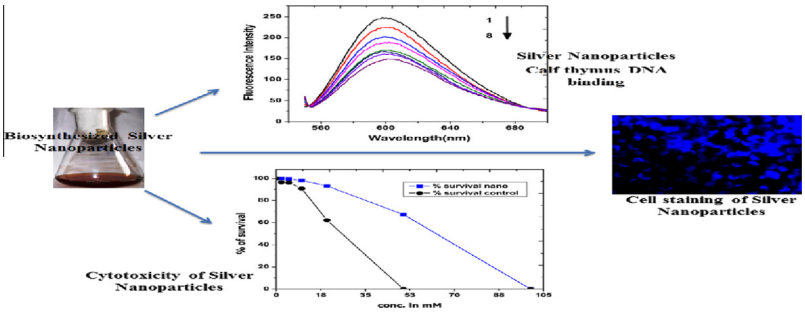
Department of Biochemistry and Biophysics, University of Kalyani, Kalyani 741235, West Bengal, India

HIGHLIGHTS

- Interaction between CTDNA and biosynthesized SNP.
- Groove binding with partial intercalation of SNP to CTDNA.
- Cytotoxicity and cell staining of SNP on HeLa cell line.

GRAPHICAL ABSTRACT

Interaction between biosynthesized silver nanoparticles and calf thymus DNA and study of silver nanoparticles toxicity as well as cell staining ability has been carried out in this work.



ARTICLE INFO

Article history:
Received 6 August 2014
Received in revised form 8 January 2015
Accepted 14 January 2015
Available online 29 January 2015

Keywords:
Silver nanoparticle
Interaction
Calf thymus DNA
Cytotoxicity
Fluorescence
Staining

ABSTRACT

The interaction of calf thymus DNA (CTDNA) with silver nanoparticles (SNP) has been investigated following spectroscopic studies, analysis of melting temperature (T_m) curves and hydrodynamic measurement. In spectrophotometric titration and thermal denaturation studies of CTDNA it was found that SNP can form a complex with double-helical DNA and the increasing value of T_m also supported the same. The association constant of SNP with DNA from UV–Vis study was found to be 4.1×10^3 L/mol. The fluorescence emission spectra of intercalated ethidium bromide (EB) with increasing concentration of SNP represented a significant reduction of EB intensity and quenching of EB fluorescence. The results of circular dichroism (CD) suggested that SNP can change the conformation of DNA. From spectroscopic, hydrodynamic, and DNA melting studies, SNP has been found to be a DNA groove binder possessing partial intercalating property. Cell cytotoxicity of SNP was compared with that of normal silver salt solution on HeLa cells. Our results show that SNP has less cytotoxicity compared to its normal salt solution and good cell staining property.

Introduction

Nanotechnology has attracted significant attention in the scientific community ever since its emergence as a powerful tool of basic and applied science [1,2]. Simultaneously, people are increasingly exposed to various kinds of manufactured nanoparticles [3,4]. Nanoparticles have widespread biological applications such as being employed in therapeutics, used as antimicrobial agents, drug delivery agents, biosensors and fluorescent labels [4,5]. Due to their unique size, nanoparticles could show highly specialized physicochemical properties, and thereby it may impose extraordinary hazards to human health and the environment [3], as a result

* Corresponding author. Tel.: +91 (033) 25828750, 2582 8378x385, +91 9046546632 (Mobile); fax: +91 33 25828282.
E-mail addresses: swaruproy@klyuniv.ac.in, swaruproy2013@gmail.com (S. Roy).

http://dx.doi.org/10.1016/j.saa.2015.01.041
1386-1425/

nano toxicology research should be carried out extensively during the days ahead.

Deoxyribonucleic acid has an important role as it carries hereditary information and instructs molecular machinery to conduct the biological synthesis of proteins and enzymes through the process of replication and transcription in living cells. Studies on the interactive mechanism of some small molecules and DNA have been identified as one of the key topics in drug–DNA interaction now-a-days [6]. Small molecules can react with DNA via covalent or non-covalent interactions, with profound interest generally focusing on the latter. There are several sites in the DNA molecule where such binding can occur: (i) between two base pairs (full intercalation), (ii) in the minor groove, (iii) in the major groove, (iv) on the outside of the helix and (v) electrostatic binding [7].

Silver nanoparticles are widely used because of their particular optical, magnetic, electronic, and catalytic properties and the same particles are also being used increasingly in wound dressings, and various family products due to their antimicrobial activity. They also serve an important function by virtue of their anti-inflammation, antivirus, anti-AIDS, and especially anticancer activities [5,8,9]. In spite of the wide usage of SNP, reports of toxicity of biosynthesized SNP are very scanty.

The interaction of DNA with metallic nanoparticles is a topic of prime interest to researchers involved in the interdisciplinary field of nano science. Studies on interaction of metal nanoparticles with nucleic acids are remarkable in the bioinorganic field due to its possible influence on the synthesis, replication, and structural integrity of DNA and RNA [10]. There are two important principal modes by which different compounds can be bound noncovalently to DNA molecule such as – intercalation and minor groove binding [11].

Nanoparticles are of similar size to typical cellular components and proteins, and thus may bypass natural mechanical barriers, possibly leading to adverse tissue reaction [12]. For nanoparticles finding its route into the clinical field, it is necessary that nanotoxicology research discovers and understands how these multiple factors influence the toxicity of nanoparticles, so that their undesirable properties can be avoided [4,13].

In the present study an attempt has been taken to investigate the interaction of SNP with calf thymus DNA by following the methods – UV spectrophotometry, fluorescence measurement, dynamic viscosity measurements, melting temperature measurement, and circular dichroism spectroscopy. We also observed cytotoxic effect of biosynthesized SNP on eukaryotic cell line (Human cervical cancer cell line, HeLa) as a model and as SNP has its own fluorescence we also take a look at the effect due to its cell staining property.

Materials and methods

Materials

Silver nitrate, Tris–base, and EDTA were purchased from Merck, Germany and ethidium bromide (EB), calf thymus DNA (CTDNA), MTT, and DMSO were purchased from Sigma Chemicals, USA. All the media, fetal bovine serum, antibiotics for cell culture were purchased from HiMedia, India. Other fine chemicals and fluorescent dye Hoechst were purchased from SRL India, and Sigma Aldrich. Other molecular biology grade fine chemicals were purchased from SRL, India. All the other chemicals were of analytical reagent grade and double distilled water was used throughout.

Apparatus

Fluorescence spectra were recorded on Agilent carry eclipse fluorescence spectrophotometer well equipped with a thermostatically peltier compartment and the excitation and emission slits were considered as 5 nm. The absorption spectra were obtained from a Cary spectrophotometer (Agilent) well equipped with a thermostatically peltier compartment. Circular dichroism was recorded in JASCO J-815 CD spectrometer and viscosity was measured in Brookfield micro viscometer. All the images were taken in Carl Zeiss Axio vision 2 fluorescence microscopes. Thermo MULTISKAN ES micro plate reader was used to measure the absorbance in the study of cytotoxicity.

Methods

Synthesis and characterization of SNP

Silver nanoparticles have been synthesized by using the cell filtrate of the fungal species, *Aspergillus foetidus* and aqueous solution of silver nitrate (at a 1 mM final concentration). The biosynthesized SNP were characterized by using several biophysical techniques such as UV–Vis spectra, Dynamic light scattering, Fourier transform infrared spectra, atomic force microscopy, transmission electron microscopy etc. as mentioned in our previous report [14]. Green synthesis is an eco-friendly, cost effective as well as an alternative way of chemical synthesis. In biosynthetic process addition of reducing agent and capping agent are not required to confer stability to the biosynthesized nanoparticles as extracellular protein alone performs the aforesaid task. Here the extracellular live cell filtrate extracted from the fungus has been used as the source of protein. After processing the estimation of concentration of biosynthesized nanoparticle has also been performed, as described in our earlier publications [15].

Spectrophotometric measurements

The CTDNA solution was prepared by dispersing an appropriate amount of CTDNA in Tris–EDTA buffer (0.1 M) solution (pH = 7.0) with stirring for 12 h at below 4 °C. The interaction between CTDNA and SNP was studied by following the measurement of UV–Vis spectra obtained in an Agilent Cary spectrophotometer. Absorption experiments were carried out by keeping constant DNA concentration 50 μg/ml whereby varying the SNPs concentration (10–80 μM). Spectral changes of DNA (50 μg/ml) were monitored after adding different concentrations of SNP (10–80 μM) by recording the UV–Vis absorption in the range of 200–400 nm. All experiments were carried out in Tris–EDTA buffer (0.1 M), pH 7.0, in a conventional quartz cell thermostatted for maintenance of the temperature at 298 K. Absorption spectrum has also been studied under the same condition after adding different concentrations of CTDNA (50 μg/ml) keeping constant SNP concentration (300 μM) by recording the spectra in the range of 300–600 nm.

Fluorescence measurements

Fluorescence measurements were carried out in an Agilent spectrofluorimeter. The solution of EB was prepared by dissolving EB in deionized water. EB was an efficient probe widely used in biochemical research for visualizing nucleic acids and it was used to assay the interaction of biosynthesized SNP with CTDNA. The fluorescence of EB is remarkably enhanced after intercalation of EB in between the base pairs of DNA [16]. The assay of ethidium bromide displacement was performed as reported in the literature [17]. At first, DNA (50 μg/ml) was added to 10 μg/ml aqueous EB solution and maximum quantum yield for EB was achieved at 270 nm, so this wavelength has been selected as the excitation radiation for samples at 293, 303, and 313 K in the emission range of 550–700 nm. To the solution containing EB and DNA different concentrations (10–70 μM) of SNP were added successively.

Circular dichroism (CD) spectral measurement

CD spectra were recorded in a JASCO J-815 spectrometer. Circular dichroism spectra showed changes in the structure of