Carbohydrate Polymers 137 (2016) 390–401

Contents lists available at ScienceDirect

Carbohydrate Polymers

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

Carbohydrate polymer inspired silver nanoparticles for filaricidal and mosquitocidal activities: A comprehensive view

Swadhin K. Saha[a], Priya Roy[b], Prasanta Saini[b], Maloy K. Mondal[a], Pranesh Chowdhury[a,*], Santi P. Sinha Babu[b]

[a] Polymer & Nano Research Laboratory, Department of Chemistry, Visva-Bharati University, Santiniketan 731235, India
[b] Parasitology Laboratory, Department of Zoology, Visva-Bharati University, Santiniketan 731235, India

ARTICLE INFO

Article history:
Received 29 September 2015
Received in revised form 2 November 2015
Accepted 3 November 2015
Available online 10 November 2015

Keywords:
Carbohydrate polymer
Silver nanoparticle
Filaricidal
Mosquitocidal
ROS
Apoptosis

ABSTRACT

The carbohydrate polymer inspired silver nanoparticles (AgNPs) are designed and synthesized through ultrasound assisted green process using unique combination of a biomolecule (tyrosine) and a natural polymer (starch). A comprehensive mechanistic study on the reactive oxygen species (ROS) mediated filaricidal (against *Setaria cervi*) and mosquitocidal (against second and fourth instar larvae of *Culex quinquefasciatus*) activities of AgNPs has been made for the first time for controlling filariasis by taking care of both filariid and its vector. The mechanism may help in formulating antifilarial drug based on carbohydrate polymer inspired AgNPs. The role of carbohydrate polymer in inspiring bioactivity of AgNPs has been looked into and its activities have been compared with the commercially available AgNPs. Cytotoxicity of AgNPs on macrophages of Wistar rat has been evaluated to ensure its selectivity towards filariid and larvae.

1. Introduction

The design and synthesis of appropriate nanomaterials with proper biomedical as well as pharmaceutical applications is one of the major breakthroughs in the field of nanomedicine. Among the various engineered nanomaterials silver nanoparticles have achieved considerable attention due to its commercial applications in the field of biomedical nanotechnology (antimicrobial effect) (Krishna Rao, Reddy, Lee, & Kim, 2012; Pinto et al., 2009; Shankar & Rhim, 2015; Travan et al., 2009; Vimala, Samba Sivudu, Murali Mohan, Sreedhar, & Mohana Raju, 2009). The antifilarial efficacy (Saha, Chowdhury, Saini, & Sinha Babu, 2014; Singh, Goswami, Sharma, Reddy, & Dash, 2012), anticancer activity (Guo et al., 2013; Vasantha, Ilangoa, Mohan Kumara, Agrawala, & Dubey, 2014; Wu et al., 2008), cellular imaging (Sokolov et al., 2003; Thurn et al., 2007), anti-HIV activity (You et al., 2012) of AgNPs are the new research fields. Singh et al. (2012) reported the microfilaricidal activity of AgNPs against *Brugia malayi* and projected AgNPs as a potential drug adjuvant against lymphatic filariasis. As the field continues to develop, detailed studies on the mechanistic path involved in antifilarial activity are required in order to advance nanotechnology for clinical use. In our previous paper, we have reported the strong micro as well as macro filaricidal activity of PVA capped AgNP against *Setaria cervi* (*S. cervi*) by induction of apoptosis (Saha, Chowdhury, et al., 2014). So far our literature survey has revealed that no work has been done to control filariasis by projecting AgNPs as both potential filaricidal and as a mosquito larvicidal agent. The beauty of the present controlling process is that it involves killing the disease causing parasite and blocking transmission of the parasite.

Recently, carbohydrate polymers (such as chitosan, cellulose and starch) are being used as a safer alternative green reagent, to synthesize the silver nanoparticles (Raveendran, Fu, & Wallen, 2003) as well as in wide range of synthesis of various compounds (Kumar, Verma, & Jain, 2015; Nasir Baig, Vaddula, Gonzalez, & Varma, 2014; Verma, Bras, Jain, & Muzart, 2013a; Verma, Bras, Jain, & Muzart, 2013b; Verma, Jain, & Sain, 2011; Verma, Tripathi, et al., 2013). Generally the carbohydrates significantly enhance the cellular uptake of AgNPs and at the same time lower the toxicity of AgNPs (Sur, Cam, Kahraman, Baysal, & Culha, 2010). Among the natural polymers, starch (a carbohydrate polymer) has been selected for designing and synthesis of desired AgNPs with the expectation that the biocompatible polymer may tune the hydrophobic and hydrophilic character of the nanoparticles. The well balanced hydrophobic and hydrophilic character along with its structural feature (linear amylose and branched amylopectin with many hydroxyl groups) is extremely helpful in penetrating cell wall.

* Corresponding author.
E-mail address: pranesh_02@yahoo.co.in (P. Chowdhury).

http://dx.doi.org/10.1016/j.carbpol.2015.11.007
0144-8617/

The ultrasound, microwave and light induced synthesis is considered as an environment friendly alternative to the conventional heating and stirring method (Bang & Suslick, 2010; Bansal, Kumar, Kumar, et al., 2015; Bansal, Kumar, Sharma, Ray, & Jain, 2015; Chowdhury, Saha, Guha, & Saha, 2012; Saha, Chowdhury, et al., 2014; Saha, Das, Chowdhury, & Saha, 2014). The ultrasound assisted synthesis provides several advantages such as milder conditions, shorter reaction times and higher yields, and produces non agglomerated nanoparticles.

Lymphatic filariasis is a neglected tropical disease which results from infection with mosquito-borne lymphatic-dwelling nematodes namely *Wuchereria bancrofti*, *B. malayi* and *Brugia timori*. *Culex quinquefasciatus* (*C. quinquefasciatus*) is the primary vector of this disease in India and it transmits the infective third-stage larvae to human. We used bovine filarial parasite *S. cervi* as model filariid. *S. cervi* resembles human filariid *W. bancrofti* in its nocturnal periodicity and antigenic variations. Easy availability of *S. cervi* makes it a good model in the field of filarial drug development. According to the World Health Organization (WHO), about 120 million people in 81 countries are infected by the disease (WHO, 2009). Approximately 40 million people suffer from morbid clinical pathology (characterized by swelling of the scrotal area and lower limbs). An estimated 1.34 billion people live in areas where filariasis is endemic and are at risk of infection (WHO, 2009). Various strategies have been applied for the eradication of this mosquito transmitted disease. Effective control of mosquito vector is a preventive approach to eliminate lymphatic filariasis as well as other serious mosquito borne diseases. But most of the marketable chemical insecticides, used to control the mosquito population, have created many ecological problems and have also eliminated beneficial organisms which consequently harm human beings (Amer & Mehlhorn, 2006). The other problems associated with the use of these chemical insecticides are the development of resistance in target vectors. These limitations urge the development of a cheap, effective and environment-friendly insecticide capable of controlling this vector. In this regard green silver nanoparticles could provide an alternative solution. Recent works have shown that the AgNPs did not exhibit any considerable toxicity for humans after *in vivo* oral exposure (at the dosage of 10 and 32 ppm) although detectable amount of silver ion was found in treated human serum (Munger et al., 2014). Travan et al. (2009) had synthesized non cytotoxic silver nanoparticle polysaccharide nanocomposites which displayed a very effective bactericidal efficacy without any cytotoxic effect towards three different eukaryotic cell lines. The *in vivo* study in rats have also revealed that no changes in haematological, body weight, food consumption, or water intake parameters have occurred in case of the AgNPs treated rats (at a dose of 30 mg/kg for 90 days) (Xia et al., 2006).

The present paper reports a complete study on design, green synthesis and the potential filaricidal and mosquitocidal activity of starch stabilized silver nanoparticles. A sincere effort has been made for the first time to elucidate the mechanism involved in biological activity and the role of the starch in their bioactivity. The cytotoxicity of the synthesized silver nanoparticles was tested on the peritoneal macrophages of Wistar rat in order to pave the way for clinical trial in the future.

2. Materials and methods

2.1. Chemicals and reagents

Starch (soluble), silver nitrate, sodium hydroxide and dimethyl sulfoxide (DMSO) and tris saturated phenol were purchased from Merck (Mumbai, India) and used as received. L-Tyrosine hydrochloride, silver nanoparticles (20 nm particle size, 0.02 mg/ml in aqueous sodium citrate buffer, it is denoted here as $AgNP_c$), foetal bovine serum (FBS), 4-(2-hydroxyethyl)-1 piperazineethanesulfonic acid (HEPES) buffer, streptomycin, penicillin, amphotericin-B, Hoechst 33258, Krebs–Ringer bicarbonate buffer, TRI Reagent, N-acetyl-L-cysteine (NAC) and 2′,7′-dichlorofluorescein diacetate (H_2DCFDA) were purchased from Sigma–Aldrich Co. (St. Louis, USA). RPMI-1640, DMEM, NBT (Nitroblue tetrazolium) and MTT (3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl-tetrazolium bromide) were purchased from Hi-Media Laboratories (Mumbai, India). Proteinase-K, DNase I and DNase free RNase were purchased from Fermentas, USA. About 60 mm plates for parasite culture were purchased from Tarsons (India). Lysis buffer (Tris–HCl 20 mM, EDTA 50 mM, SDS 0.5%, NaCl 100 mM, β mercaptoethanol 1% (v/v), pH 8.0), phosphate buffer saline (PBS), TE buffer was prepared in our laboratory.

2.2. Instrumentation

A Sonicator (Branson-1510) was used to obtain ultrasound of 42 kHz. The UV–vis spectra were recorded by Shimadzu UV spectrophotometer (UV-1800). Particle size, selected area electron diffraction (SAED) and the energy-dispersive X-ray (EDX) spectrum were recorded using high resolution transmission electron microscope (HRTEM) (JEOL JEM-2100, 200 K.V., Japan). The Field emission scanning electron microscope (FESEM) imaging was carrying out by ZEISS SUPRA-40 (Germany) Instrument. Zeta potential and dynamic light scattering (DLS) study was done by Malvern instrument. Microtiter plate reader (Beckman, USA) was used to measure the optical density of formazan crystals. Inverted fluorescence microscope (Dewinter, Victory, Italy) and gel documentation system (Bio-Rad, USA) were used for photography and agarose gel electrophoresis respectively. Fluorescence spectrometer of (Perkin Elmer LS 55) was used for determination of reactive oxygen species (ROS).

2.3. Synthesis of starch stabilized silver nanoparticle

Different amount of starch (Table 1) was dissolved in 40 ml of hot Millipore water with the help of sonication. Then, 10 ml of $AgNO_3$ (different concentrations) solution was added to the starch solution gradually from a micro-burette. The mixture was subjected to sonication for 5 min. Then the different amount of 10^{-3} M alkaline tyrosine solution was added drop wise to the former solution. The resulting mixture was subjected to sonication for 30 min.

2.4. Filaricidal and mosquitocidal activities of the silver nanoparticles

2.4.1. Collection of adult and microfilariae of S. cervi

Adult female worms of *S. cervi* with average length of 5.5 ± 1.0 cm and average weight of 30.0 ± 5.0 mg were retrieved from the peritoneal cavity of freshly slaughtered cattle and placed in a vacuum flask containing Krebs–Ringer's solution. After bringing to laboratory, worms were washed with the Ringer's solution to free them from any extraneous material and placed in humidified incubator (37 °C) consisting of 5% CO_2 for reviving the motility of parasites. Microfilariae (mf) were collected by dissecting the gravid female of *S. cervi*.

2.4.2. Collection and maintenance of mosquito larvae in laboratory condition

We had collected the second and fourth-instar larvae of *C. quinquefasciatus* from standing water of cemented drains surrounding the campus of Visva-Bharati University (23.68° N and 87.68° E). The larvae were placed in a plastic enamel tray containing tap water along with glucose and yeast powder. They were maintained in the