Enzyme and Microbial Technology 92 (2016) 18–25

Contents lists available at ScienceDirect

Enzyme and Microbial Technology

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

Trypsin inactivation by latex fabricated gold nanoparticles: A new strategy towards insect control

Chandrashekhar D. Patil [a], Hemant P. Borase [a], Rahul K. Suryawanshi [a], Satish V. Patil. [a,b,*]

[a] School of Life Sciences, North Maharashtra University, Post Box 80, Jalgaon, 425001, Maharashtra, India
[b] North Maharashtra Microbial Culture Collection Centre (NMCC), North Maharashtra University, Post Box 80, Jalgaon, 425001, Maharashtra, India

ARTICLE INFO

Article history:
Received 2 February 2016
Received in revised form 10 May 2016
Accepted 9 June 2016
Available online 11 June 2016

Keyword:
Proteases
Trypsin
Plant latex
Gold nanoparticles
Enzyme inactivation
Insect control

ABSTRACT

Before applying nanotechnologies in biomedical and environmental areas it is advised to study interactions of nanoparticles and other nanomaterials with biomacromolecule present in living system. Moreover there is scarcity of reports on interactions between nanoparticles and biomaterials. In present report a rapid, ecofriendly method of fabricating stable gold nanoparticles (AuNPs) using latex of *Jatropha curcas* is reported for the first time. AuNPs found to have characteristic absorption maxima centered at 540 nm, multiple irregular shapes with size range from 20 to 50 nm and have crystalline nature. Latex fabricated AuNPs were found to inhibit catalytic potential of trypsin (a vital enzyme responsible for digestion, insecticide resistance and in several disease conditions). The interactions between AuNPs and trypsin were analyzed by UV–vis spectrophotometry and microwave plasma-atomic emission spectrometry which suggests formation of trypsin-AuNPs complex responsible for lowering catalytic activity of trypsin. Transmission electron microscopy, Fourier transform infrared spectroscopy and particle size distribution studies further confirm complex formation between trypsin and AuNPs. Diverse interactions of metal nanoparticles with proteins such as covalent interaction, electrostatic interactions and binding to —SH group of amino acid may be the reasons behind inhibition of trypsin activity. In vivo studies on serum of several vectors and agriculturally important pests supported instrumental results on AuNPs induced trypsin inhibition. This work will bring a new research direction to explore eco-friendly nanoparticle in insect control via inhibition of enzyme catalytic potential.

1. Introduction

Among different nanomaterials gold nanoparticles (AuNPs) have emerged as an important candidate in diverse fields such as optoelectronics, cosmetics, imaging, new generation nanosensor, tissue engineering, drug delivery and biocidal applications [1]. AuNPs was also analyzed in past for their potential to act as vehicle for targeted delivery of drugs (drug carrier) and other important biomolecules such as DNA/RNA, enzymes, proteins due to its wide desirable properties such as stability, non-toxicity, biocompatibility, wide size range (1–150 nm) making possible to attached diverse molecules on its nanosurface [2]. But, before applying biomacromolecule loaded AuNPs in living system or in biomedical applications it is necessary to study the interactions of biomacromolecule with nanoparticles with respect to change in chemistry and effects on the function/performance of molecules attached on nanosurface as such type of studies will reveals fate of molecules attached on nanosurface in biological systems [3]. Several studies have been emphasized on chemistry between nanoparticles and biomolecules and its effects on alternation in activities of enzymes, proteins and drugs [4].

Regulation of enzyme activity in biological system has vital importance due to indispensible role of enzyme's in variety of biological functions ranging from digestion to replication. Trypsin is a serine protease (EC 3.4.21.4). It is a proteolytic enzyme having molecular weight of 23.3 kDa, found in digestive tract of animals, insects and microorganisms [5,6]. Trypsin is associated with some disorders such as platelet aggregation, cystic fibrosis, pancreatitis, acute abdominal disorders [7–9]. Apart from its prime role in hydrolysis of peptide bonds of proteins, trypsin found to play an important role in insect world by detoxifying xenobiotics (insecticides) [6,10].

Vector-bourne diseases caused pandemic and epidemic consequences in developing countries of tropical and subtropical region. Every year more than one billion people are infected and around one million dies from vector-borne diseases [11]. Insects such as mosquitos (*Aedes aegypti*, *Anopheles stephensi*) are responsible for

* Corresponding author at: School of Life Sciences, North Maharashtra University, Post Box 80, Jalgaon, 425001, Maharashtra, India.
E-mail address: satish.patil7@gmail.com (S.V. Patil.).

http://dx.doi.org/10.1016/j.enzmictec.2016.06.005
0141-0229/

transmission of diseases like malaria, filariasis, dengue, chikungunya and Japanese encephalitis accounting global mortality and morbidity [12].

Insecticides of different classes such as organophosphates, organochlorine, carbamate are majorly used to control mosquito population in urban areas. As a result of intense selection pressure mosquitoes continue to develop resistance against insecticides in many ways, namely target side mutation, enzyme modification, sequestration, quick elimination [10]. Overproduction of resistance associated enzymes is also a tool used by insect to protect themselves from insecticides. Recently, Tetreau et al. found an increase in proteolytic activities in dengue fever mosquito in response to insecticide [6].

The term latex was coined in early of 1600 by an English physician, when he observed similarity of latex extrusion system of plants with lymphatic vessel of animals carrying blood [13]. Plant latex is a sticky emulsion of different color but is mostly of milky white color and present in about 10% (about 20,000) flowering plant species [14]. Latex protects plant from herbivores by different mechanisms such as toxicity, anti-nutritive effects, trapping of insect due to its sticky nature. Previous studies on plant latex were performed with respect to their role in plant-insect interaction, biochemical consortium of novel metabolites (fibrinolytic protease, anticancer phenol) and pharmacological properties. *Jatropha* curcas (Family-Euphorbiaceae) is latex producing plant having economically importance in biodiesel production and have many medicinal properties and biological activities (nematicidal, fungicidal, larvicidal and insecticidal activities) [15]. In present study latex of *J. curcas* is exploited for the first time as eco-friendly reducing and stabilizing agent for synthesis of AuNPs.

Nanoparticles can be an ideal candidate for inactivating mosquitoes and other insect enzymes associated with insecticide resistance (trypsin and other protease) because of its small size, specific interactions with active site amino acids and potential to alter structural configuration of enzymes [3,4,16]. So, in present study trypsin inactivation caused by green synthesized AuNPs is elaborated. Spectroscopic studies using *N*-benzoyl-D,L-arginine-*p*-nitroanilide (BAPNA) as substrate to analyze in vitro trypsin activity inhibition and serum protease analysis of mosquito and other insects as in vivo measurement of trypsin inactivation was performed. UV–vis spectroscopy, Fourier transform infrared spectroscopy, transmission electron microscopy, particle size analysis and microwave plasma-atomic emission spectrometry techniques ware utilize to reveal mechanism behind AuNPs induced trypsin inhibition. This is the first report explaining inhibition of insect protease using latex (bio) fabricated AuNPs.

2. Experimental

2.1. Materials

Gold chloride trihydrate ($HAuCL_4{\cdot}3H_2O$), *N*-benzoyl-D,L-arginine-*p*-nitroanilide (BAPNA) was purchased from Sisco Research Laboratory, Mumbai, India and stored at −20 °C. Trypsin (from porcine pancreas), bovine serum albumin, *p*-nitroaniline (p-NA) and Tris buffer (pH7.8) were obtained from HiMedia Lab, India. Ultra-pure water was used in all experiments.

3. Methods

3.1. Synthesis of AuNPs

10 mL aqueous solution of gold chloride (0.1 mM) was mixed with 20 μL freshly collected latex (colorless and slightly turbid latex extrudes out from *J. gossypifolia* by cutting near young leaves and stems were collected in sterile eppendorf tube) followed by 5 min incubation at room temperature. Reaction mixture was observed for ruby red color formation as it is the primary indication of AuNPs synthesis.

3.2. Characterization of AuNPs

Ecofriendly synthesized AuNPs were characterized by different analytical tools according to our earlier published reports [12,17]. After addition of aqueous gold chloride salt solution to latex, AuNPs synthesis was monitored periodically for visual color change in reaction mixture. UV–vis spectra operated at 200–700 nm were used for preliminary examination of nanoparticles synthesis by analyzing presence of characteristic surface plasmon resonance (SPR) absorbance (Shimadzu 1601 spectrophotometer, Shimadzu, Japan). Transmission electron microscopy (TEM PHILIPS, CM 200, Operating voltages: 20–200 kv, Netherland) was done to reveal size and shape of nanoparticles. Selected area electron diffraction (SAED) analysis informed about crystalline nature of nanoparticles. Analysis for presence of elemental gold in sample was carried by energy dispersive X-ray analysis (EDAX) analysis (X-Max, 80 mm2; Oxford instrument). X-ray powder diffraction analysis was done on XRD- Brucker D8 Advance, Karlsruhe, Germany with measurement of diffraction intensity between 20–80^0 2 Ø angle. XRD gives information about crystallinity of AuNPs. Distribution of different size particles dispersed in AuNPs solution and stability of AuNPs were interpreted from dynamic light scattering and Zeta potential analysis (Zeta sizer Malvern Instruments, Westborough, MA, U.S.A.). After confirmation of nanosynthesis, the AuNPs were subjected to Fourier transform infrared (FT-IR, IR Prestige-21, Shimadzu, Japan) spectroscopy to find role of latex biomolecules in present on surface of nanoparticles and involved in formation and stabilization of nanoparticles.

3.3. Trypsin inhibition study (Trypsin- AuNPs interactions)

3.3.1. Interaction between trypsin and AuNPs

Study on interaction between AuNPs and the most important protease (trypsin) was analyzed using UV–vis spectroscopy between wavelength range of 210–800 nm (Shimadzu 1601 spectrophotometer, Shimadzu, Japan). Interaction experiment was performed by mixing and incubating (1 h) different volumes of as synthesized latex fabricated AuNPs with trypsin (1000 μg/mL) at optimum conditions (pH-8, temperature 37 °C). Assay protocol involves addition of 500 μL trypsin with 100, 200, 300 and 500 μL AuNPs. Absorption spectra of trypsin alone and after incubation of trypsin with AuNPs were recorded [18,19].

3.3.2. N-*benzoyl*-D,L-*arginine*-p-*nitroanilide (BAPNA) experiment and thin layer chromatography (TLC)*

N-benzoyl-D,L-arginine-*p*-nitroanilide (BAPNA) is a colorless synthetic substrate for trypsin. Stock solution of *N*-benzoyl-D,L-arginine-*p*-nitroanilide was prepared by dissolving 5 mg of *N*-benzoyl-D,L-arginine-*p*-nitroanilide into 2 mL dimethylsulfoxide and then diluted to 50 mL with tris buffer (100 μg/mL). Trypsin degrades *N*-benzoyl-D,L-arginine-*p*-nitroanilide into *p*-nitroaniline, a yellow color product having maximum absorbance at 365 nm. In a control experiment *N*-benzoyl-D,L-arginine-*p*-nitroanilide was subjected to trypsin (500 μL *N*-benzoyl-D,L-arginine-*p*-nitroanilide + 500 μL trypsin) and as according to its nature trypsin cleaved *N*-benzoyl-D,L-arginine-*p*-nitroanilide into *p*-nitroaniline. The absorbance of *p*-nitroaniline at 365 nm was noted. In test, trypsin was mixed with AuNPs (100 μL) and incubated for 2 h and then this trypsin- AuNPs mixture was mixed with *N*-benzoyl-D,L-arginine-*p*-nitroanilide followed by measurement of absorbance at 365 nm. The percent inactivation of trypsin