Food Chemistry xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Food Chemistry

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

Sensitivity enhancement for mycotoxin determination by optical waveguide lightmode spectroscopy using gold nanoparticles of different size and origin

Nóra Adányi[a,*], Ádám György Nagy[a], Bettina Takács[a], István Szendrő[b], George Szakacs[c], Rózsa Szűcs[d], Eszter Tóth-Szeles[d], István Lagzi[d,e], Diána Weiser[c], Viktória Bódai[c], Péter Sátorhelyi[c], Balázs Erdélyi[c]

[a] Food Science Research Institute, National Agricultural Research and Innovation Centre, Herman Ottó út 15, H-1022 Budapest, Hungary
[b] MicroVacuum Ltd., Kerékgyártó u. 10, H-1147 Budapest, Hungary
[c] Fermentia Microbiological Ltd, Berlini utca 47-49, H-1045 Budapest, Hungary
[d] Budapest University of Technology and Economics, Department of Physics, Budafoki út 8, H-1111 Budapest, Hungary
[e] MTA-BME Condensed Matter Research Group, H-1111 Budafoki út 8, Budapest, Hungary

ARTICLE INFO

Chemical compounds studied in this article:
Aflatoxin B1 (PubChem CID: 186907)
AuNP (PubChem CID: 23985)
Tetrachloroauric acid trihydrate (PubChem CID: 44134746)
Tetramethylammonium hydroxide (PubChem CID: 44134746)
Dichloromethane (PubChem CID: 6344)
11-Mercaptoundecanoic acid (PubChem CID: 543502)
γ-Aminopropyltriethoxysilane (PubChem CID: 13521)
N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (PubChem CID: 15908)
N-hydroxysuccinimide (PubChem CID: 80170)
Ethanolamine hydrochloride (PubChem CID: 74819)

Keywords:
BioAuNPs
Aflatoxin B1
Immunosensor
Spice paprika
OWLS

ABSTRACT

Mycotoxins, present in a wide range of food and feed commodities, are toxic secondary metabolites produced by a number of different fungi. Certain mycotoxins do not readily degrade at high temperatures, therefore are resistant to food processing, and consequently are present in the human and animal food supply.

Optical waveguide lightmode spectroscopy (OWLS) was applied for the detection of aflatoxin B1, in a competitive immunoassay format, to compare the analytical sensitivity achieved with an immunosensor design allowing signal enhancement by increasing the sensor surface through immobilization of gold nanoparticles (AuNPs) of different size and origin (obtained by chemical or biotechnological synthesis). The effects of AuNPs median size, the methods of sensitization and the biochemical parameters on immunosensor performace were examined. After optimization of the sensitized sensor surface, an immunosensing method was developed for the analysis of aflatoxin in paprika matrix and the results were compared with HPLC reference measurements.

1. Introduction

Nanotechnology and nanomaterials have important roles in several fields, such as environment protection, medicine and the chemical and food industries. Precious metal nanoparticles, particularly gold nanoparticles (AuNPs) can be used as drug delivery systems, catalysts or biosensors, due to their biocompatibility, vast surface area and stable chemical and physical properties. Traditionally, AuNPs of various sizes, polydispersity and shapes have been synthesized by wet chemical methods but, in many cases, these methods are of high energy demand and environmental loads (Jana, Gearheart & Murphy, 2001; Xiao & Qi, 2001). Chemical and physical dispersion methods have several disadvantages, such as the use of harsh chemicals and synthesis conditions and formation of toxic residues. Therefore, there is an increasing

* Corresponding author.
E-mail addresses: n.adanyi@cfri.hu (N. Adányi), a.gy.nagy@cfri.hu (Á.G. Nagy), szucs.rozsa@mail.bme.hu (R. Szűcs), lagzi@nimbus.elte.hu (I. Lagzi), peter.satorhelyi@fermentia.hu (P. Sátorhelyi), Balazs.Erdelyi@fermentia.hu (B. Erdélyi).

https://doi.org/10.1016/j.foodchem.2018.04.089
Received 20 February 2017; Received in revised form 31 March 2018; Accepted 21 April 2018

demand for environmentally friendly synthetic methods. Biological methods offer nontoxic and sustainable synthesis procedures (Mohanpuria, Rana & Yadav, 2008). These green biotechnological processes – synthesis of nanoparticles by plant extracts, fungi or bacteria – could overcome the environmental harm (Vágó et al., 2016) associated with existing chemical methods. Several microorganisms, particularly mesophilic and thermophilic fungi appear promising for the production of nanoparticles and the properties of these AuNPs can be fine-tuned through optimization of fermentation processes.

One of the most important applications of AuNPs is in biosensor development (Chauhan et al., 2016; Salam, Uludag & Tothill, 2013). Isolated bio-AuNPs with well-defined physical and chemical properties, after cleaning and surface modifications, can be applied as promising amplifiers in real-time quartz crystal microbalance (QCM) sensors for sensitive and rapid detection of aflatoxin (e.g. in cereals, spice paprika), which is important in numerous agricultural processes as well as food safety (Adányi et al., 2007; Wang, Niessner, Tang & Knopp, 2016). Immobilization of AuNPs on the biosensor surface increases the specific surface area and the covalent attachment of AuNPs to the antibody causes weight increase, amplifying the biosensor signal.

Mycotoxins are toxic secondary metabolites produced by a number of different fungi. Among mycotoxins aflatoxin B1 (AFB1) occurs most commonly, produced primarily by Aspergillus strains (Sudini et al., 2015). It is also the most toxic mycotoxin, composed of a very stable coumarin moiety, and therefore its monitoring and control in foodstuffs is required (Bennett & Klich, 2003; Hussein & Brasel, 2001).

Fungi can colonize and contaminate food and feed before harvest or during storage, following prolonged exposure to high humidity or damage under stressful conditions. Certain mycotoxins are resistant to food processing and do not decompose at high temperatures, contaminating human and animal food supplies (Hildebrand et al., 2015; Liu, Zhou & Shi, 2015). Thus, mycotoxin contamination is one of the most common problems regarding food safety (Li, Liu & Lin, 2012). According to a survey by the Food and Agriculture Organisation (FAO), mycotoxins contaminate about 25% of agricultural products around the world each year causing a variety of toxic effects in humans and animals (FAO, 2001).

The aim of this study was to investigate the potental of enhanced sensitivity using chemically and biologically synthesized AuNPs for an OWLS immunosensor applied in model measurements using BSA – anti-BSA immunocomplex. After optimization a novel method was developed for the detection of aflatoxin in paprika and the results compared with the OWLS sensing method without the application of AuNPs and with HPLC reference measurements. The method developed could be used for various food or feed constituent; and spice paprika was selected for use in this study to demonstrate that the method is applicable in complex and difficult food matrices as well.

2. Materials and methods

2.1. Materials

AFB1 from *Aspergillus flavus* used as immunogen, BSA- and AFB1-specific polyclonal antibodies, AFB1-BSA conjugate used as a coating antigen, AuNP standards with PEG 3000 and carboxylic end groups, tetrachloroauric acid trihydrate ($HAuCl_4 \times 3H_2O$), tetramethylammonium hydroxide, dichloromethane, 11-mercaptoundecanoic acid, γ-aminopropyltriethoxysilane (APTES), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), ethanolamine hydrochloride and other chemicals were purchased from Sigma-Aldrich Co. LLC (St. Louis, MO, USA) unless otherwise stated. The bioAuNPs were produced as detailed below.

Hungarian paprika samples were treated with different *Aspergillus* mold strains were investigated to artificially induce aflatoxin contamination.

2.2. Preparation, functionalization and purification of biogold nanoparticles (bioAuNPs)

AuNPs were synthesized using a biological method (Vágó et al., 2016). For synthesis a gold salt (tetrachloroauric acid trihydrate – $HAuCl_4 \times 3H_2O$) as the precursor and a fungal cell-free extract from thermophilic fungi in an acetate buffer environment (pH 4.6) were used. The cell-free extract (shake flask fermentation supernatant) was prepared from different fungal strains and fermentation was carried out on an orbital shaker at 28 °C for 3 days. The fermentation broth was centrifuged and the supernatants were used for the biosynthesis. Synthesis was carried out at 45.0 ± 0.5 °C.

The biologically synthetized AuNPs (8.0 mL, $\sim 10^{-3}$ M in terms of gold atoms) and tetramethylammonium hydroxide (0.8 mL, 1.0 M) was mixed and placed in a vessel with a solution of 11-mercaptoundecanoic acid (8.0 mL, 10^{-3} M) in dichloromethane. The two-phase system was stirred for 20 h at room temperature. Then, the organic phase was removed, and the water phase was washed three times with 4.0 mL of dichloromethane to remove the excess of 11-mercaptoundecanoic acid. The AuNPs were precipitated by the addition of hydrochloric acid (4.0 mL, 0.1 M). After centrifuging the formed precipitate, the liquid phase was removed and the solid phase was washed three times with hydrochloric acid (8.0 mL, 0.1 M) to prevent dissolution of the AuNPs. After purification, aggregated AuNPs were redispersed in water by the addition of a few drops of 1.0 M tetramethylammonium hydroxide.

Table 1 summarizes the different type of AuNPs used for the measurements.

2.3. Optical waveguide lightmode spectroscopy

OWLS measurements were performed using amino-functionalized integrated optical waveguide sensors (chips) of type OW2400 (MicroVacuum Ltd., Budapest, Hungary) on a glass support and with a fine optical grating in the waveguide layer. The sensor output was read with an OWLS 210 instrument controlled by BioSense 3.7 (MicroVacuum Ltd., Budapest, Hungary). When a polarized laser beam (He–Ne laser, 632.8 nm) reaches the grating, it is diffracted, and the diffracted beam, when incoupled into the waveguide, propagates toward the edge of the sensor through multiple internal reflections. The intensity of the incoupled light is measured with a photodiode. By detection of the incoupling angle, changes in the refractive index can be followed in real time due to the association of the analyte molecules at the sensor surface. All the experiments were performed in a flow-injection analyzer (FIA) system containing an ESA model 582 solvent delivery module (ESA Biosciences Inc., Chelmsford, MA, USA) HPLC pump with a flow rate of 160 μL min^{-1} and an injector (Rheodyne, Rohnert Park, CA, USA) equipped with a sample loop of 200 μL. The sensor holder was temperature-controlled by an OWLS TC heater/cooler unit (MicroVacuum Ltd., Budapest, Hungary).

Table 1
The origin and the median particle size of the applied gold nanoparticles.

	Origin of AuNP	Median size
A	**Without AuNP**	**–**
B	Sigma 765430	5.0 nm
C	Sigma 765538	15.0 nm
D	Sigma 765473	30.0 nm
E	*Humicola insolens* CBS 147.64	76.5 nm
F	*Rhizopus pusillus* WFPL 267A (ATCC 16458)	60.1 nm
G	*Thermoascus aurantiacus* TUB F-43 (ATCC 58156)	54.2 nm
H	*Thermomucor indicae-seudaticae* NRRL 6429 (ATCC 28404)	69.5 nm
I	*Thielavia terrestris* NRRL 8126 (ATCC 38088)	124.0 nm