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Investigation of biocatalytic enlargement of gold nanoparticles using dynamic light scattering and atomic force microscopy

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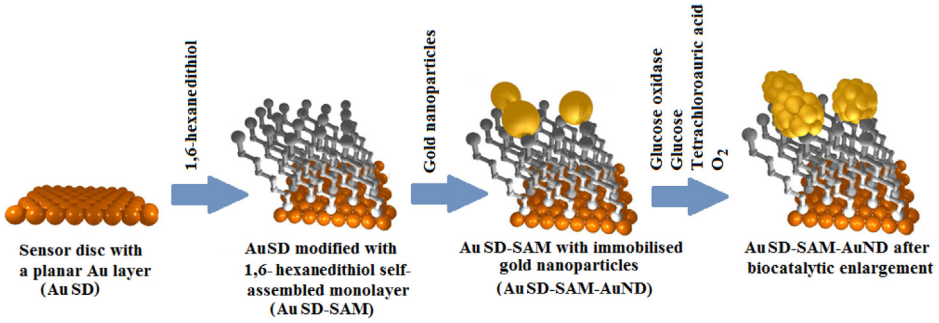
HIGHLIGHTS

- The biocatalytic enlargement of AuNPs using GOx was investigated by UV–vis spectroscopy, DLS and AFM.
- The optimal rate of AuNPs and growing solution components was determined.
- AuNPs were investigated in the solution and immobilized on 1,6-hexanedithiol modified sensor disc with planar gold layer.
- The plasmon absorbance, hydrodynamic diameter and height of enlarged AuNPs changes depending on the concentration of glucose and duration of enlargement.

GRAPHICAL ABSTRACT



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ABSTRACT

The aim of this study was the investigation of the biocatalytic enlargement of colloidal gold nanoparticles (AuNPs) using enzyme glucose oxidase (GOx), glucose and tetrachloroauric acid by UV–vis spectroscopy, dynamic light scattering (DLS) and atomic force microscopy (AFM). The appropriate ratio of 13 nm AuNPs seeds and growing solutions equal to 1:2 was determined from absorbance spectra of AuNPs. At this ratio the most AuNP seeds were biocatalytically enlarged and it resulted in the higher difference of plasmon absorbance after 60 and 90 min of AuNPs incubation in growing solution. In order to determine the size of AuNPs after biocatalytic enlargement, DLS measurements in the solution and AFM measurements after the immobilization of AuNPs seeds on sensor disc coated with a planar Au layer, which was functionalized with 1,6-hexanedithiol self-assembled monolayer, were performed. The DLS and AFM measurements showed that hydrodynamic diameter of biocatalytically enlarged AuNPs in the solution and the height of nanoparticles immobilized on the surface depends on the glucose concentration in the solution and on the duration of synthesis. Therefore, the biocatalytic enlargement of AuNPs could be applied in the development of glucose biosensors using UV–vis spectroscopy, DLS and AFM methods.

1. Introduction

Nowadays gold nanoparticles (AuNPs) have been applied in many areas such as electronics, photonics, catalysis, chemical and biochemical sensing [1–3]. The inherent property of AuNPs to catal-

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yse the reduction of gold ions on the already existing AuNPs seeds was successfully applied for the optical detection of NAD(P)H cofactor [4], glucose, cholesterol, neurotransmitters, tyrosine and others substances [5–7]. The mechanism of AuNP seeds enlargement in the solution containing tetrachloroauric acid ($HAuCl_4$) and H_2O_2 has been studied in detail. It was shown that AuNP seeds acted as catalysts for the reduction of Au^{3+}, which is involved into $AuCl_4^-$ anion, by H_2O_2 on the surface of nanoparticles forming layer of Au^0-based nanocrystallites [8].

The application of enzymes for the enlargement of AuNP seeds and enhancement of analytical signal is a very promising direction in analytical chemistry [6,7,9]. Different oxidases, which are producing H_2O_2 during enzymatic reaction, can be successfully applied for this purpose. The enlargement of AuNPs immobilized on the glucose oxidase (GOx) was used for the improvement of the electrical contact of redox enzyme with the electrode and for the enhancement the electron transfer in the ferrocene mediated system [7]. Colorimetric glucose biosensor based on the H_2O_2 mediated enlargement of AuNPs immobilized on the glass substrate was demonstrated. The increase of the plasmon absorbance was observed by increasing glucose concentration in reaction solution [8]. The system with AuNPs seeds adsorbed onto the surface of gold electrode modified by self-assembled monolayer (SAM) could be applied for the determination of both H_2O_2 and cholesterol while using cholesterol oxidase as biocatalyst. As a result, the AuNPs seeds were enlarged and the peak currents in cyclic voltammograms were inversely proportional to the concentration of analytes [9]. Moreover, electrochemical immunosensors, which were dedicated for protein biomarker detection based on enlarged and surface charged AuNPs mediated electron transfer, were designed [10]. Advantages of optical DNA sensor developed for the registration of hybridization event using catalytic growth of AuNPs were discussed [9]. Additionally, synthesis of AuNPs at 37 °C in the presence of glucose and GOx was used for the colorimetric quantification of analyte. In this method increased concentration of glucose resulted the increase of plasmon absorbance, which is related to the increased concentration of AuNPs [11]. Additionally, AuNPs could be synthetized by γ-irradiation using water-soluble chitosan and enlarged by adjusting the ratio of $Au^{3+}/Au^0_{(seeds)}$ [12].

The size of synthetized AuNPs mainly is determined using UV–vis spectroscopy, dynamic light scattering (DLS) measurements, atomic force microscopy (AFM) and transmission or scanning electron microscopy (TEM or SEM). AFM was successfully explored for the study of AuNPs growth kinetics using classical Turkevich citrate synthesis method at two different temperatures. In addition, the size of synthetized AuNPs was determined using well established methods dedicated for the particles characterization such as DLS and TEM [13].

The aim of this study was the investigation of the biocatalytic enlargement of AuNPs using GOx, glucose and $HAuCl_4$. The enlargement of AuNPs at optimal conditions changing concentration of glucose was studied by three methods – UV–vis spectroscopy, AFM and DLS methods. The dependence of nanoparticle size on the concentration of glucose was investigated.

2. Materials and methods

2.1. Materials

1,6-hexanedithiol [$HS(CH_2)_6SH$] (1,6-HDT), methanol, hydrochloric acid, potassium hydrogen phosphate, disodium hydrogen phosphate, tannic acid, and trisodium citrate dihydrate were obtained from Sigma-Aldrich (Germany). Glucose oxidase from *Aspergillus niger* (EC 1.1.3.4, type VII, 215 U/mg). D-(+)-glucose, tetrachloroauric acid trihydrate ($HAuCl_4 \times 3\ H_2O$) and tannic acid were obtained from Carl Roth GmbH&Co (Germany). All other chemicals used in the present study were either of 'analytically pure' or of 'highest grade' quality. Surface plasmon resonance sensor disc with a planar Au layer (Au SD) were received from XanTec bioanalytics GmbH (Germany). The solution of glucose was prepared at least 24 h before the use in order to allow glucose to mutarotate and reach equilibrium between α and β forms. The AuNPs growing solution was composed of 25 μg mL^{-1} GOx, 0.976 mM $HAuCl_4$ and corresponding concentration of glucose in 0.01 M phosphate buffered saline (PBS) pH 6.0.

2.2. Synthesis of AuNPs

AuNPs were synthesized reducing $AuCl_4^-$ anions by sodium citrate in the presence of tannic acid as an additional reductant. 80 mL of 0.0125 % [w/v] of $HAuCl_4$ aqueous solution and a mixture of 20 mL consisting of sodium citrate (4 mL of 1% [w/v]) and tannic acid (0.025 mL of 1 % [w/v]) in deionised water were prepared [14,15].These solutions in deionised water were heated in an Erlenmeyer flasks up to 60 °C on a magnetic stirrer with electric heating. After preheating solutions were mixed, heated up to 98 °C and kept at this temperature for 3 min to yield solution of nearly monodispersed AuNPs of 13.0 nm in diameter that was determined by AFM [16]. Solution of AuNPs was stored in dark glass flasks at +4 °C.

2.3. Biocatalytic enlargement of AuNPs in the solution

Colloidal AuNPs and 0.01 M PBS solution were mixed at different ratios maintaining the same final concentration of GOx (25 μg mL^{-1}) and $HAuCl_4$ (0.976 mM) (growing solution) in order to determine the optimal composition for the biocatalytical enlargement of AuNPs (Fig. 1A). Six ratios of colloidal AuNPs and growing solution (1:1, 2:3, 1:2, 1:3, 1:6 and 1:14) with 4×10^{-2} M of glucose were analysed at 20 °C temperature. The appropriate ratio of AuNPs and components needed for the enlargement of AuNPs was determined spectrophotometrically using UV–vis spectrophotometer Lambda 25, PerkinElmer (Shelton, USA). The position and the high of absorbance spectra maximum depend on particle size and concentration.

2.4. Preparation of Au SD surface for AuNPs immobilization

A bare sensor disc surface was cleaned with 1 M NaOH solution for 20 min and then with 1 M HCl solution for 5 min. After the rinsing with deionised water and drying, the sensor disk was transferred to 1 mM 1,6-HDT in methanol and stored 24 h to form a self-assembled monolayer (SAM) of the alkane dithiols. After the rinsing out the excess of alkane dithiols firstly with methanol and then with water, and drying the 1,6-HDT-modified sensor disc was used for AuNPs immobilization.

2.5. Biocatalytic enlargement of AuNPs immobilized on the surface

Sensor disc functionalized with 1,6-HDT exposing thiol groups on the surface was placed into colloidal solution of AuNPs for 20 min and then washed by water. The strong interaction between sulphur and gold atoms leads to the 13 nm AuNPs immobilisation on the surface. The enlargement of AuNPs was performed in the growing solution for 60 and 90 min changing glucose concentration (Fig. 1B).

2.6. Evaluation of enlarged AuNPs size

The DLS method was used for the evaluation of AuNPs hydrodynamic diameter after biocatalytic enlargement. Samples for DLS measurements were prepared by mixing of AuNPs with solution

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