International Journal of Biological Macromolecules 51 (2012) 998–1002

Contents lists available at SciVerse ScienceDirect

International Journal of Biological Macromolecules

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

Structural changes of glucose oxidase upon interaction with gold-coated magnetic nano-particles

Khadijeh Eskandari [a], Hedayatollah Ghourchian [a,b,*]

[a] Laboratory of Microanalysis, Institute of Biochemistry & Biophysics, University of Tehran, P.O. Box 13145-1384 Tehran, Iran
[b] Nanobiomedicine Center of Excellence, Nanoscience and Nanotechnology Research Center, University of Tehran, Tehran, Iran

ARTICLE INFO

Article history:
Received 17 September 2011
Received in revised form 31 July 2012
Accepted 1 August 2012
Available online 9 August 2012

Keywords:
Glucose oxidase
Gold coated magnetic nanoparticles
Structural change

ABSTRACT

Structural changes of glucose oxidase (GOx), as a typical protein, upon interaction with gold-coated magnetic nano-particles (GMNPs) were studied. GMNPs were characterized by UV–vis, FTIR and SEM. Due to GMNPs interaction, obvious changes in enzyme conformation and its secondary and tertiary structures were verified using intrinsic fluorescence and circular dichroism. Moreover, Zeta potential measurement showed GMNPs surface charge shift to more negative value due to interaction with GOx. Finally, the electrochemical results revealed that while the enzyme electron transfer rate on GMNPs is higher, this process on cysteamin–GMNPs is more reversible.

1. Introduction

In recent years, considerable attention has been focused on immobilization of enzymes on nano-particles for the applications of biotechnological processes such as biosensors and fuel cells. For a successful immobilization the parameters such as surface properties of nano-particles, the functional groups on protein surface and the distance between nano-particles and protein molecules should be considered [1,2]. Gold nano-particles are expected to exhibit attractive properties because of their small size, high surface area and good biocompatibility [3]. Nowadays, the magnetic nano-particles receive increasing attention due to their application in biotechnology. For example by using an external magnetic field magnetic nano-particles could be applied for separation of various biomolecules from a reaction mixture [4,5]. By reducing the nano-particle size, not only the reactivity of the particles increases but also large amount of protein is loaded due to increasing the specific binding surface area. The magnetic nanoparticles are usually covered by either a non-metallic or a metallic layer. Therefore, magnetic nano-particles coated by metals such as gold is common due to the ease of synthesis and their chemical functionality [6]. Nevertheless, in most cases the proteins conjugated to the nano-particles faced with structural changes [7]. This, in practice, will make a limitation for applicability of protein.

One of the important proteins is glucose oxidase (GOx) which is currently receiving much attention due to its wide applications in chemical, pharmaceutical, food and clinical industries. Also, this enzyme is extensively used in biotechnology and recently along with nano-particles in designing novel glucose biosensors. However, application of proteins beside nanoparticles might alter their conformation or structure [8]. Therefore, it seems that there is an increasing interest in understanding and controlling the interactions between nanomaterials and biological molecules such as enzymes [9].

In the present work GOx, as a typical protein, was used to consider its conformational and structural changes due to interaction with gold-coated magnetic nano-particles (GMNPs). A variety of methods including circular dichroism (CD), Fourier transform infrared (FTIR), fluorescence and UV–vis spectroscopy has been used for characterization of GMNPs and their interaction with GOx. Also the electrochemical method was used to study the changes in protein electron transfer.

2. Experimental

2.1. Reagents and apparatus

$FeCl_2{\cdot}7H_2O$, $FeCl_3{\cdot}6H_2O$, NaOH, HCl, $HAuCl_4{\cdot}3H_2O$, tetramethylammonium hydroxide pentahydrate ($TMAOH{\cdot}5H_2O$), horse radish peroxidase (HRP), o-dianisidine, glucose and glucose oxidase were purchased from Sigma–Aldrich (Saint Louis, MO, USA). Hydrogen tetrachloroaurate ($HAuCl_4{\cdot}3H_2O$), cysteamine, trisodium citrate, potassium dihydrogen phosphate (KH_2PO_4) and

* Corresponding author at: Laboratory of Microanalysis, Institute of Biochemistry & Biophysics, University of Tehran, P.O. Box 13145-1384 Tehran, Iran.
Tel.: +98 216640 8920; fax: +98 21 6640 4680.
E-mail address: hadi@ibb.ut.ac.ir (H. Ghourchian).

0141-8130/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2012.08.001

dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Merck (Darmstadt, Germany) and used as received. The electrochemical experiments were carried out using a computerized Potentiostat/Galvanostat (model 263-A, EG&G, USA) equipped with Power Suite software package. Electrochemical studies were performed using a single-compartment conventional three-electrode cell at room temperature. A working modified polycrystalline gold-disk electrode with a diameter of 3 mm, a saturated silver/silver chloride (Ag/AgCl) reference electrode, containing 3 M KCl (from Azar Electrode Co., Iran), and a platinum rod auxiliary electrode were used. All potentials were measured and reported vs. the Ag/AgCl reference electrode. Circular dichroism spectroscopy was done with Aviv, model 215 spectropolarimeter (Lakewood, NJ, USA). Fluorescence spectroscopy was carried out using Hitachi spectrofluorimeter (MPF-4 model, Japan). UV–vis spectroscopy was performed by Cary spectrophotometer (100 Bio-model, USA). Fourier transform infrared (FTIR) spectra were recorded using Fourier transform infrared spectrometer (Model Nexus 870, Thermo Nicolet Co., USA). Scanning electron microscopic (SEM) images were obtained using a Scanning electron microscope (Model LEO 440i, UK). For Zeta potential measurement, Zeta potential analyzer was used (Zeta Plus, Brookhaven Instruments Corporation, USA).

2.2. Preparation of the gold-coated magnetic nanoparticles

GMNPs were prepared following the procedure described in the literature [10]. In short, 5.4 g $FeCl_2 \cdot 7H_2O$ and 2.0 g $FeCl_3 \cdot 6H_2O$ were dissolved in 25 mL of 10 mM HCl and the solution was drop-wised to 250 mL of 1.5 M NaOH solution under vigorous stirring. A black precipitate immediately formed. It was washed with distilled water to remove the excess NaOH, and then dried at 60 °C. In order to encapsulate the prepared magnetic iron nanoparticles with a gold shell, 5 mL of iron oxide nanoparticles were suspended in 0.1 M TMAOH (pH 12) solution and added to 95 mL of citric acid (5 mM) and stirred vigorously. Into the magnetite solution, 1% $HAuCl_4 \cdot 3H_2O$ were added until the solution became purple in color.

2.3. Immobilization of GOx on GMNPs

Two strategies for GOx immobilization were designed. In the first one, 12 mg of GMNPs were suspended in 500 μL of phosphate buffer (0.1 M, pH 6.8) solution (PBS), and then 20 μL of GOx (10 mg/mL) was added to the solution. During this process GOx directly chemisorbed on nanoparticle. After 2 h, the adsorbed GOx on GMNPs were collected by a permanent magnet and the additional GOx was removed by washing. Thereafter, the GOx/GMNPs were fixed on a gold plate electrode using a permanent magnet. The magnet was kept under the gold plate. In the second strategy, 100 μL of 0.01 M cysteamine was added to 500 μL of suspended GMNPs. In this process cysteamine molecules are self-assembled on nanoparticles through thiol groups [11]. After 20 min, cysteamine/GMNPs were collected by an external magnet and the additional cysteamine was removed by washing. Then 20 μL of GOx (10 mg/mL) was added to 500 μL of cysteamine/GMNPs solution for 30 min and after removing the additional enzyme by washing, GOx/cysteamine/GMNPs were fixed on a gold plate electrode by a permanent magnet.

3. Results and discussion

3.1. Scanning electron micrograph

Fig. 1 shows the SEM image of GMNPs. It reveals that the GMNPs are spherical with the diameter less than 50 nm.

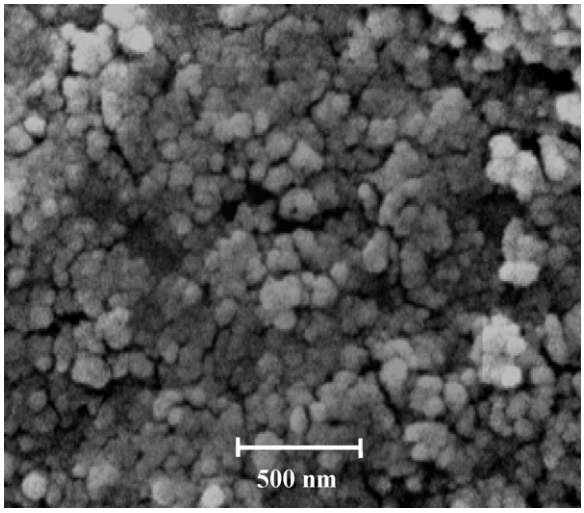

Fig. 1. SEM images of gold magnetic nano-particles.

3.2. Zeta potential measurements

Zeta potential (ξ) is the electrostatic potential, which is related to both surface charge and the local environment of the particle. Using Zeta Potential Analyzer, Zeta potential of naked GMNPs and GOx/GMNPs were recorded at 25 °C while the particles were suspended in water. Zeta potential could automatically be calculated from electrophoretic mobility based on the Smoluchowski equation:

$$\nu = \left(\frac{\varepsilon E}{\eta}\right)\xi \quad (1)$$

where ν is the measured electrophoretic velocity, η is viscosity, ε is the electrical permittivity of the electrolytic solution and E is the electric field [12]. The Zeta potential of GMNPs was determined as -36.29 ± 1.96 mV ($n = 5$). However, by absorbing GOx on GMPNs, the Zeta potential shifted to -44.94 ± 1.67 mV ($n = 5$). Such a shift could be due to the enzyme surface charge. Since the isoelectric point of GOx is 4.2, at pH 6.8 the net surface charge of GOx would be negative. This causes the Zeta potential of GOx/GMPNs shifted to more negative charges relative to that of GMNPs.

3.3. UV–vis spectroscopy

The UV–vis absorption spectra of gold nanoparticles (GNPs), magnetic nanoparticles (MNPs) and GMNPs are shown in Fig. 2. As seen in (spectrum a), no absorption peak was observed for the black magnetic nanoparticles but the red color GNP colloids showed an absorption peak at 526 nm (spectrum b) and GMNP colloids with a black-red color showed a broad spectrum with a maximum absorption at 538 nm (spectrum c). The red shift and broadening in the surface plasmon absorption of GMNP colloids relative to the GNP colloids revealed that the size distribution of GNPs is relatively narrower than that of the GMNPs and therefore possibility for aggregation of GMNPs is more serious than the GNPs [13].

Since the UV–vis spectroscopy is sensitive to the possible change in the molecule environment, therefore this technique was used to study the interaction between GOx and nanoparticles. Fig. 3 shows the UV–vis absorption spectra of GMNPs (a), GOx (b) and the mixture of GOx and GMNPs (c) in 0.1 M PBS, pH 6.8. As seen, GMNPs exhibited a distinct surface plasmon absorption peak at 539 nm and GOx showed the characteristic peak of peptide bound at 273 nm. But the mixture of GOx and GMNPs showed two distinct peaks at 540 and 273 nm for GMNPs and protein, respectively. This revealed that GOx was absorbed on the GMNPs.