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The Effect of Hydrophobicity and Hydrophilicity of Gold Nanoparticle on Proteins Structure and Function

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ABSTRACT

The surface parameter of nanoparticles such as hydrophobicity and a hydrophilicity on protein structure and function is very important. In this study, conformational changes of glucose oxidase (GOx) in the mercaptopurine: GNPs and 11-mercaptoundecanoic acid: GNPs as a hydrophobic and a hydrophilic GNPs surface was investigated by various spectroscopic techniques, including: UV-Vis absorption, fluorescence and circular dichroism (CD) spectroscopies. Moreover, the fluorescence quenching constant and binding parameters after the formation of the GOx: GNPs conjugates follows by Stern-Volmer (S-V) plots. Size of GNPs was determined by Zeta Sizer, which their size is 80 nm. CD and florescence spectroscopy show that the conformational changes in both the secondary and the tertiary structure levels of GOx in conjugate with hydrophobic and hydrophilic-GNPs was occured. Also, Stern-Volmer plots for the binding of hydrophilic-GNPs and hydrophobic-GNPs with GOx was plotted. Stern-Volmer quenching constant, binding constant and the number of binding sites of GOx: GNPs conjugates was determined.

Keyword: Glucose oxidase (GOx); Hydrophobic-GNPs; Hydrophilic-GNPs; Fluorescence and circular dichroism (CD) spectroscopy.

1. INTRODUCTION

Nanoparticles have a great role in diagnostic, drug delivery, therapy, biosensing and so on. Therefore, it is necessary to improve the knowledge of the mechanisms of nanoparticles interaction with proteins, cells and tissues, for applying to the design of applicable nanodevice. In drug delivery; its necessary nanoparticles are having at least tend to protein. Because when bound to proteins, they may be quickly cleared by macrophages before they can reach target cells [1] and sometimes such as biosensors and bio-fuel cells, high ratio of absorption of protein is needed. Therefore, the study of

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nanoparticle surface for protein immobilization and protein biocompatibility is required. Also, the changes in the structure and function of protein caused to the thoughtful effects biological activity or the activation of immune response [2, 3].

Among the various methods to characterize the protein conformational changes, the spectroscopic method is the most commonly adopted methods, that including circular dichroism (CD), UV-Vis spectroscopy, Fourier transform infrared (FTIR) spectroscopy, fluorescence spectroscopy, and so on [4-7].

Glucose oxidase (GOX, β-D-glucose oxygen 1-oxidoreductase, EC 1.1.3.4) is a homodimer flavoprotein containing two active sites per molecule [8-9]. It catalyses the oxidation of β-D-glucose to gluconic acid, concomitant with the reduction of oxygen to hydrogen peroxide. Glucose oxidase as a cheap and available enzyme has been used to test various types of enzyme immobilization, and is the most commonly studied in the construction of biosensors for glucose assay development [10].

In this study, colloidal gold nanoparticles (NC-GNPs) was synthesized by a popular procedure [11]. Also the hydrophobic and hydrophilic GNPs was prepared by mercaptopurine and 11-mercaptoundecanoic acid respectively. Then the effect of nanoparticle coating on protein structure and function as well as protein adsorption were studied.

2. EXPERIMENTAL

2.1. Reagents

$HAuCl_4 \cdot 3H_2O$ and glucose oxidase, Horse radish peroxidase (HRP), o-dianisidine, glucose, mercaptopurine and 11-mercaptoundecanoic acid 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 dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Merck (Darmstadt, Germany) and used as received.

2.2. Apparatus

Circular dichroism spectroscopy was done with Aviv, model 215 spectropolarimeter (Lakewood, NJ, USA), fluorescence spectroscopy with Hitachi spectrofluorimeter (MPF-4 model, Japan) and the UV-Vis spectroscopy by Cary spectrophotometer (100 Bio-model, USA). The hydrodynamic size and the surface charge (zeta potential) of nanoparticle were characterized with a Zeta sizer and Zeta potential analyzer (Zeta Plus, Brookhaven Instruments Corporation, USA).

2.3. Gold nanoparticles synthesis and conjugation forms

For preparation of colloidal gold nanoparticles (GNPs), 25 mL of 0.01% (w/v) $HAuCl_4 \cdot 3H_2O$ were heated up to 60°C, then 2 mL of 0.1% (w/v) sodium citrate added to it. The final red color nanoparticles was stored in dark glass bottles at 4°C [12]. For preparation of hydrophobic and hydrophilic GNPs, 10 mM of mercaptopurine and 11-mercaptoundecanoic acid was added to GNPs with the ratio of 1/20 respectively.

3. RESULTS AND DISCUSSION

3.1. Size and surface charge analysis

The hydrodynamic size and the surface charge (zeta potential) was investigated by dynamic light scattering (DLS) and electrophoretic light scattering (ELS), respectively [13]. The hydrodynamic size of GNPs is 80 nm and their surface charge is -16 mV. Also, the concentration of GNPs is calculated to be approximately 3.5×10-15, assuming that all gold in the $HAuCl_4$ was reduced.

3.2. Characterization of the synthesized GNPs and GOx/GNPs conjugates

The GNPs solution exhibits a color of dark red, which is known to arise from the collective oscillation of the free conduction electrons induced by an interacting electromagnetic field. UV-Vis absorption measurements indicated that the maximum wavelength of the surface plasmon resonance (SPR) was 533 nm (Figure 1). The position of this

peak is almost unchanged in all of the GNPs, but the shape of the peak is different, especially in the hydrophobic GNPs, showing that the hydrophobic surface is susceptible to agglomeration. But hydrophilic GNPs interact with the aqueous surroundings and remain separate particles in solution.Also, in comparison with the peak of the GNPs, the peak intensity of GOx/GNPs conjugates is significantly reduced, indicating that the GOx was binding on the GNPs surface.

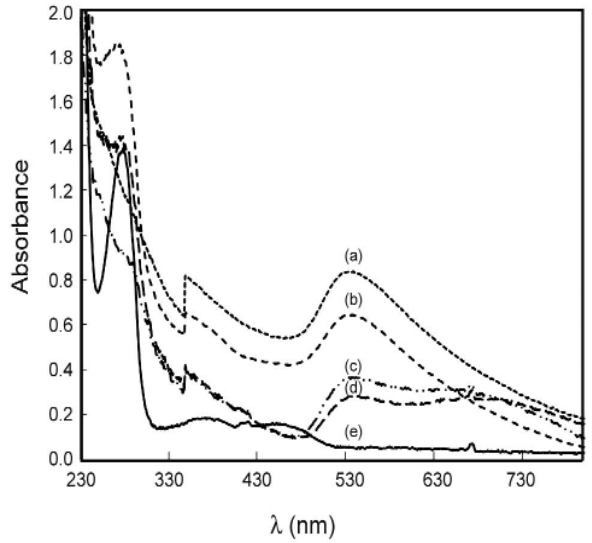

Figure 1: *UV-Vis spectra of hydrophilic-GNP (a), GOx: hydrophilic-GNP conjugates (b), hydrophobic-GNP (c), GOx: hydrophobic-GNP conjugates (d) and GOx (e). 20 μL (8 mg/mL) GOx in 200 μL GNPs.*

Moreover GOx exhibits an absorbance maximum in 280 nm, which originates from peptide bonds and aromatic residues. And in GOx/GNPs, this peak shift to 270 nm, which confirms the GOx binding on the GNPs too.

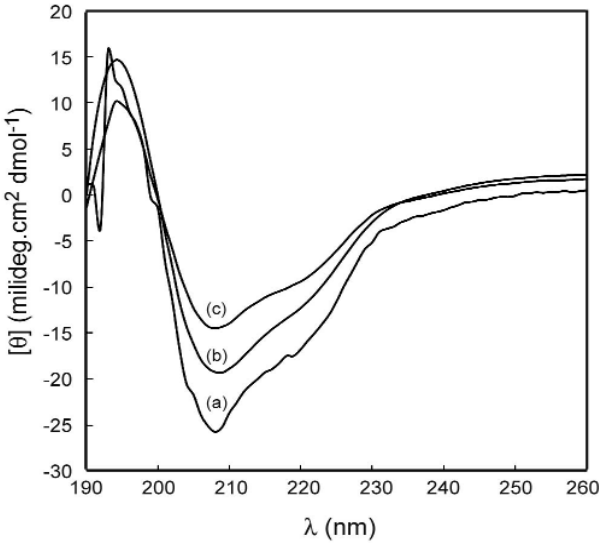

Figure 2: *CD spectra of GOx, 5 μL GOx (30 mg/mL) solutions was added to 245 μL PBS (52 mM), pH 7.4 (a) and the conjugates of GOx: hydrophilic-GNPs (b) and GOx: hydrophobic-GNPs (c). 5 μL (30 mg/mL) GOx solution was mixed with 100 μL GNPs and was diluted by 145 μLPBS (52 mM), pH 7.4.*

3.3. Circular dichroism spectroscopy

CD spectroscopy is one of the useful and common methods to study of protein conformations in solution or adsorbed onto colloidal surfaces. CD spectroscopy was performed for investigation of secondary structure in GOx/GNPs conjugates. Figure 2 shows the far-UV CD spectra of native

Table 1: *Secondary structures percentage of GOx on different GNPs was obtained by deconvolution.*

secondary structures	GOx Native	GOx: hydrophilic -GNPs	GOx: hydrophobic -GNPs
α-helix	28.9	22.1	17.8
β-sheet	21.3	31.3	32
β-turn	17.6	19.5	20
Random coil	34.8	36.7	40.6

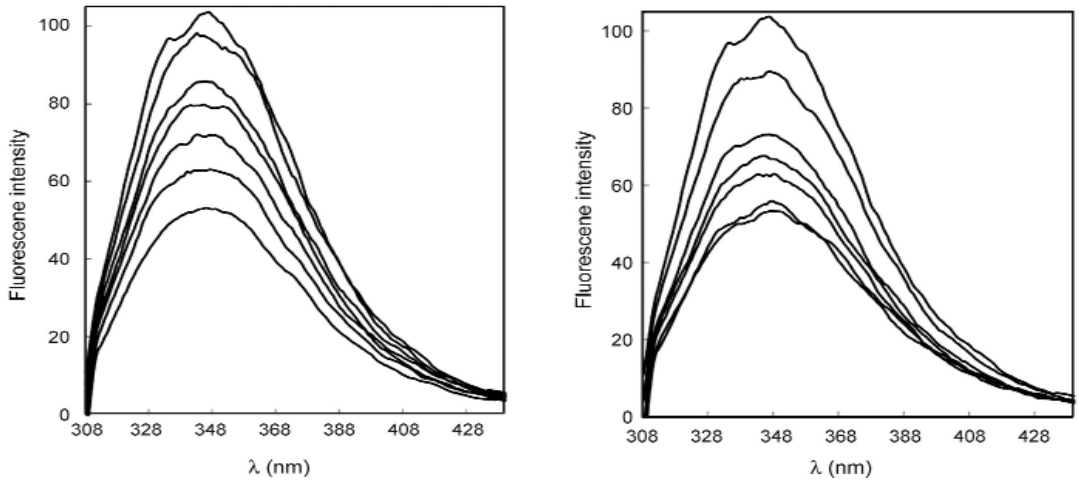

Figure 3: *Effect of hydrophilic-GNPs (A) and hydrophobic-GNPs (A) on the fluorescence spectrum of GOx. 2 μL (30 mg/mL) GOx was added to 0, 10, 20, 40, 60, 80, 100 μL GNPs (from up to down) and the final volume was brought to 200 μL by PBS (52 mM), pH 7.4.*

GOx (a) and the conjugates of GOx: hydrophilic-GNPs (b), GOx: hydrophobic-GNPs (c). Deconvolution of the spectra reveals that the % α-Helicity in of native GOx is 28.9%, but decreases to 22.1 and 17.8% and subsequently beta structure increase from 21.3% to 31.3 and 32% for hydrophilic-GNPs and hydrophobic-GNPs respectively (Table 1). On the other hand, the conjugation of GOx on GNPs leads to alpha-beta transition [14]. However, hydrophilic-GNPs are better surface for link of protein.

3.4. Binding property of the GNPs nanoparticle to the GOx

Fluorescence spectroscopy is useful to obtain local information about the conformational changes of protein at tertiary structure levels. Typically, from the interpretation of fluorescence parameters, one can obtain information such as the degree of exposure of the fluorophore to the solvent and the extent of its local mobility. For proteins with intrinsic fluorescence, more specific local information can be obtained by selectively exciting the tryptophan (Trp) residues. GOx contains 10 Trp per each subunit, therefore any changes in the enzyme conformation and oxidation states have been proved to affect the tryptophan fluorescence of GOx [15]. Figure 3 (A and B) shows the emission spectra of native GOx at different concentrations of GNPs upon excitation at 295 nm. The choice of 295 nm as the excitation wavelength was to avoid the contribution from tyrosine residues [16]. The fluorescence intensity was found to decrease with increasing the GNPs while the emission maximum shifted from 345 nm at native GOx to 346.5 and 345.5 nm at hydrophilic-GNPs (A) and hydrophobic-GNPs (B) respectively. The shift in the position of emission maximum reflected the changes of the polarity around the Trp residues. The slightly red shifts on GNPs indicate that Trp residues are partly exposed to the solvent. Figure 3A shows the decrease in fluorescence intensity with the increase of hydrophilic-GNPs, this may be due to the fact that water molecules were placed between the enzyme and the hydrophilic-GNPs [17].

These different fluorescent characteristics reflected different conformational states of GOx on GNPs. Fluorescence intensity data were then analyzed using the Stern-Volmer equation.

$$\frac{F^0}{F} = K_{sv}[Q] + 1 \qquad \text{(Eq. 2)}$$

Where F^0 and F are the maximum fluorescence intensities in the absence or presence of quencher, respectively, KSV is the Stern-Volmer quenching constant and [Q] is the concentration of quencher. The linearity of the F0/F versus [Q] plots is shown in Figure 4. Also The binding constant (K) and the number of binding sites (n) between GNPs with GOx can be calculated using the Eq. 2.

$$Log\left[\frac{F^0 - F}{F}\right] = LogK + nLog[Q] \qquad \text{(Eq. 2)}$$

A plot of log [(F^0- F)/F] versus log [Q] gives a straight line, whose slope equals to n (the number of binding sites) and the intercept on Y-axis equals to log K [18]. The results revealed the presence of a single class of binding site on GOx. The values of KSV, K and n are listed in Table 2.

Table 2: *Stern-Volmer quenching constant, binding constant and the number of binding sites of GOx:GNPs conjugates interactions.*

No.	K_{SV} ($\times 10^{15}$ M^{-1})	K ($\times 10^{-6}$ M^{-1})	n
hydrophilic-GNPs	0.53	1.41	1
hydrophobic-GNPs	0.47	0.27	0.7

3.5. GOx absorption studies

Due to large surface area/volume ratio nanoparticles tend to high adsorb proteins. GOx concentrations absorbed on the GNPs were determined by the Bradford methods [19]. The amount of GOx adsorbed on hydrophilic-GNPs and hydrophobic-GNPs were measured to be 1.83 ± 0.03 and 1.11 ± 0.02 μg/mL, respectively. In the other word, the nanoparticle-protein ratio is one molar of nanoparticle to 3.5 and 2.1×10^6 M of GOx on hydrophilic-GNPs and hydrophobic-GNPs, respectively. Also the nanoparticle-protein surface ratios, by Assuming they are spherical, are approximately 171, indicating that the protein adsorption on the nanoparticle is multilayer.

Moreover these results clearly indicate that hydrophilic GNPs display good protein adsorption. So it can be said, although different types of forces such as hydrophobic interactions and coordination binding might also work in the conjugation of protein with nanoparticles but the electrostatic force have a highlight role. By considering the 15 lysine residue in Gox, protein binding to hydrophilic-GNPs is possible with electrostatic force too [16].

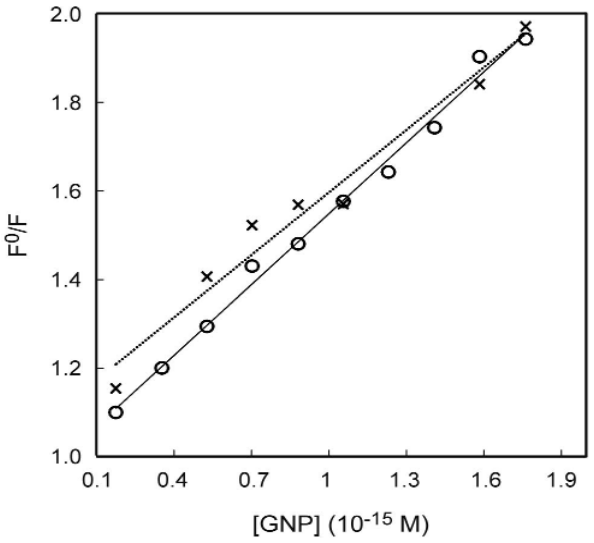

Figure 4: *Stern-Volmer plots for the binding of hydrophilic-GNPs (σ) and hydrophobic-GNPs (×) with GOx.*

3.6. Enzymatic activity measurements by reaction with a substrate

The enzymatic activity (U) represents conversion of 1 μmol of the substrate per minute and the specific activity is defined as the enzymatic activity per mg of the enzyme (U/mg) at 25°C. The activity of GOx was assayed colorimetrically by UV-Vis spectroscopy after 20 min incubation of GOx with GNPs [20]. The activity of native GOx and GOx solution in the presence of on hydrophilic-GNPs and hydrophobic-GNPs were measured to be 180, 87 and 47 U/mg, respectively. These data indicates that in the presence of hydrophilic-GNPs, the

enzyme activity conserved more than hydrophobic-GNPs.

4. CONCLUSIONS

In this work, the hydrophobic and hydrophilic GNPs was prepared by mercaptopurine and 11-mercaptoundecanoic acid respectively. Then the effect of nanoparticle coating on protein structure and function as well as protein adsorption was studied by a combination of spectroscopic techniques. In UV-Vis spectroscopy intensity of GOx/GNPs conjugates is significantly reduced, indicating that the GOx was binding on the GNPs. Moreover, CD and florescence spectroscopy show that the conformational changes in both the secondary and the tertiary structure levels of GOx in conjugate with hydrophilic-GNPs are lower than hydrophobic-GNP. Because hydrophobic-GNP is insusceptible for agglomeration and despite the lower absorption of the enzyme, but it have most conformational changes. Therefore, hydrophilic surface is most biocompatible than hydrophobic surface for protein immobilization.

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