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An efficient colorimetric biosensor for glucose based on peroxidase-like protein-Fe_3O_4 and glucose oxidase nanocomposites

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ABSTRACT

An efficient colorimetric biosensor for glucose based on peroxidase-like protein-Fe_3O_4 and glucose oxidase nanocomposites is reported in this work. Compared with bare MNPs, peroxidase-like casein-MNPs exhibit good catalytic properties, stability, dispersibility. Casein incorporated on MNPs notably improves the affinity toward both H_2O_2 and TMB, proved by variation in the determined kinetic parameters. As low as 0.2 μM H_2O_2 can be detected with a linear range from 0.5 μM to 200 μM H_2O_2. More importantly, the casein/MNP nanocomposite was further used to immobilize GO_x and to construct a glucose biosensor for the one-step determination of glucose. This method is simple, inexpensive, highly sensitive, and selective for glucose detection, with a detection limit of 1.0 μM over a linear range from 3 μM to 1000 μM.

1. Introduction

Magnetite (Fe_3O_4) particles have long been considered as one of the most attractive nanomaterials for applications in magnetic resonance imaging, biological separation, drug delivery, and biological catalysis, particularly due to their favorable magnetic properties and low toxicity (Wang et al., 2008; Laurent et al., 2008). Recently, it was reported that Fe_3O_4 magnetic nanoparticles (MNPs) exhibited an intrinsic enzyme mimetic activity similar to that found in natural peroxidases (Gao and Yan, 2007; Wei and Wang, 2008; Liu et al., 2011). Compared with a native enzyme-horseradish protein (HRP), the enzyme-like nanomaterials display potential excellent properties, such as greater resistance to extremes of pH and temperature. Furthermore, their preparation, storage, and separation are relatively simple and cheaply. However, bare MNPs often display aqueous instability, due to their magnetic attractive forces and the long-rang van der Waals attractive forces (Bonacchi et al., 2004; Lee et al., 2007). In addition, magnetic nanoparticles display much weaker affinity toward substrates than HPR (Gao and Yan, 2007), which results in low catalytic activities and restricted applications.

Detection of glucose has been paid much attention in biomedical fields and plays an increasingly important role in the improvement of life quality (Mizutani and Yabuki, 1997; Radhakumary and Sreenivasan, 2011). H_2O_2 is the main product of the glucose oxidase (GO_x)-catalyzed reaction, and the enzyme-like nanomaterials based sensors were developed for colorimetric detection of glucose (Wei and Wang, 2008; Yu et al., 2010). However, due to the different optimal pH for enzyme-like nanomaterials and GO_x, the detection of glucose by using enzyme-like nanomaterials usually involved two-step analytical processes in these studies. First, GO_x was used to catalyze the oxidation of glucose in neutral buffer. Then, the H_2O_2 produced served as a substrate for MNPs to catalyze the oxidation of the peroxidase substrate to the colored radical in acidic buffer. Obviously, the method used was a multiple-step analysis, and GO_x cannot be recycled in those studies. This limits the application of those enzyme-like nanomaterials in practice.

Considering the above limitations of the enzyme-like nanomaterials, a suitable surface functionalization plays a crucial role (Yu et al., 2009; Liu and Yu, 2011). A suitable surface modification should provide good colloidal stability of MNPs, high affinity towards the substrates of the MNPs, and functional groups to immobilize enzyme on MNPs. Recently, Yu et al. reported that SH–NH_2–MION, by virtue of its features of both sulfhydryl groups and amines, displayed elevated affinity toward substrates, and high storage stability (Liu and Yu, 2011). There is still a much requirement for searching for new functionalized materials to realize the highly sensitive sensing of hydrogen peroxide and other oxidase-based reactions giving H_2O_2 as a product (such as glucose).

Proteins with carboxyl and hydroxyl functional groups have the potential to coat magnetic nanoparticles. The conjugation of

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proteins with nanocrystals not only affords stabilization of the system, but also introduces biocompatible functionalities onto these nanoparticles for further biological application (Gole et al., 2001; Jiang et al., 2005). Casein with an isoelectric point of about 4.5 has a highly content of acid amino acid residues (such as glutamic acid and aspartic acid residues), and is negatively charged at neutral pH. The casein constituents, α_{s1}-, α_{s2}-, β-, and κ-casein, exist in proportions of approximately 4:1:4:1 by weight, and can be thought of as block copolymers consisting of blocks with high levels of hydrophobic or hydrophilic amino acid residues. Therefore, caseins exhibit a strong tendency to self-assemble into spherical casein micelles (Liu and Guo, 2007).

Herein, we report a facile synthesis of aqueous casein stabilized, monodisperse iron oxide nanoparticles via a coprecipitation method. The as-prepared casein-MNPs were then used to catalyze the oxidation of a peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) by H_2O_2 to the oxidized colored product which provides a colorimetric detection of H_2O_2. As low as 0.2 μM H_2O_2 could be detected with a linear range from 0.5 to 200 μM via our method. More importantly, a one-step, sensitive and selective method for glucose detection was developed using the composite of casein-MNPs and GO_x. At pH 5.5, the casein-MNP/GO_x nanocomposites catalyze the oxidation of glucose to generate H_2O_2. At the same pH buffer, the resulting H_2O_2 oxidizes TMB in the presence of casein-MNP/GO_x nanocomposites. The detection platforms for H_2O_2 and glucose developed in the present work confirmed that the casein-MNPs not only possess highly effective peroxidase-like activity but also provide functional surfaces suitable for immobilizing biomolecules and preserving their biological activity.

2. Experimental section

2.1. Chemicals and materials

Ferric chloride, ferrous chloride, ammonium hydroxide (25–28 wt%), and 30% H_2O_2 were purchased from Shanghai Chemical Reagent Company (Shanghai, China). Casein (isoelectric point (pI)=4.5 and average molecular weight (MW)=2.1 kDa), 3,3′,5,5′-tetramethylbenzidine (TMB), β-D-glucose, maltose, α-lactose, D-fructose, and glucose oxidase (from *Aspergillus niger*, GO_x) were purchased from Sigma-Aldrich. Other reagents and chemicals were at least analytical reagent grade. Distilled water was used as the solvent.

2.2. Preparation of MNPs, casein-MNPs and casein-MNP/GO_x nanocomposites

The Fe_3O_4 MNPs were first prepared via a previously reported coprecipitation method (Kim et al., 2001). In a typical reaction, 0.086 g $FeCl_2$ and 0.216 g $FeCl_3$ were mixed in 10 mL water and heated to 80 °C under argon in a three-necked flask. While vigorously stirring the reaction mixture, 5 mL of 25% NH_4OH was introduced by syringe, and the heating continued for 1 h. The formed black Fe_3O_4 colloidal particles were separated by using a permanent magnet and further washed with water three times. The Fe_3O_4 MNPs were then redispersed in 5.0 mg/mL casein solution under sonication for 20 min at room temperature. The MNP concentration determined using the 1,10-phenanthronline colorimetric method (Yu et al., 2010), is about 5 mg/ml.

Excess amount of GO_x (50 μL, 10 mg/mL) was added to a solution of casein-MNPs (50 μL, 5 mg/ml). The resulting mixture was incubated at ambient temperature. The particles adsorbed with GO_x were separated from nonadsorbed GO_x by using a permanent magnet. To estimate the amount of GO_x adsorbed on the casein-MNPs, the supernatant (obtained from a solution containing casein-MNPs and GO_x) was measured by UV adsorption (Bradford, 1976). The amount of GO_x immobilized on the surface of casein-MNPs (~2.5 mg/mL) was estimated to be ~2 mg/mL. The collected composites were washed two times with 20 mM phosphate buffer (pH 7.0). The immobilization strength of GO_x on casein-MNP was tested by redispersing and incubating these enzyme-covered MNPs in phosphate buffer (0.1 M and pH 7.0) at room temperature for 8 h. The dispersion was separated by using a permanent magnet, and the GO_x concentration in the supernatant was measured by UV adsorption.

2.3. Characterization

The morphologies of products were examined by a transmission electron microscopy (TEM, Tecnai-12 Philip Apparatus Co.). Fourier transform infrared (FTIR) spectra of products were recorded in the range 400–4000 cm^{-1} using FTIR spectroscopy (Nicolet-740). The sample was prepared in a pellet form with spectroscopic-grade KBr. The X-ray diffraction (XRD) patterns of samples were recorded on a German Bruker AXS D8 ADVANCE X-ray diffractometer. Ultraviolet–visible (UV–vis) spectra (UV-2501, Shimadzu Corp., Japan) of products dispersed in water were measured in the range 300–800 nm.

2.4. H_2O_2 detection using casein-MNPs as peroxidase mimetics

To investigate the peroxidase-like activity of the as-prepared casein-MNPs, the catalytic oxidation of the peroxidase substrate TMB in the presence of H_2O_2 was tested. In a typical experiment, 30 μL of 8.0 mM TMB, 30 μL of the casein-MNPs stock solution (5 mg/mL), and 30 μL of 5 M H_2O_2 were added into 2.91 mL of 0.1 M pH 4.0 acetate buffer at 35 °C. Immediately after the substrates were added, color reactions were observed. All the reactions were monitored by the adsorption spectroscopy measurement.

2.5. Glucose detection using casein-MNP/GO_x nanocomposites

Glucose detection was realized as follows: 30 μL glucose with various concentrations was added to 2.91 mL acetate buffer (0.1 M, pH 5.5) containing the casein-MNP/GO_x nanocomposites (30 μL, 2.5 mg mL^{-1}) and TMB (30 μL, 8.0 mM). The resulting solution was incubated for 30 min at 35 °C. The casein-MNP/GO_x nanocomposites were then removed from the reaction solution by an external magnetic field. The final reaction solution was used to perform the adsorption spectroscopy measurement.

A human serum sample from a local hospital was used for measuring the amount of glucose. 300 μL serum was added to 2.64 mL acetate buffer (0.1 M, pH 5.5) containing the casein-MNP/GO_x nanocomposites (30 μL, 2.5 mg mL^{-1}) and TMB (30 μL, 8.0 mM). The resulting solution was incubated for 30 min at 35 °C. The final reaction solution was used to perform the adsorption spectroscopy measurement.

3. Results and discussion

3.1. Characterization of casein-MNPs

The as-prepared casein-MNP was identified from the X-ray diffraction (XRD) patterns. The pattern of the casein-MNPs (Fig. S1, Supporting Information) shows all the major peaks corresponding to Fe_3O_4. This indicates that the presence of casein capping agent did not alter the crystalline structure of resultant Fe_3O_4 nanoparticles. Fig. S2a presents a representative TEM micrograph of casein-MNPs dispersed in water. The nanoparticles were spherical in shape with slightly polydisperse size distribution, and apparently well dispersed. However, for bare MNPs (Fig. S2b), MNPs

cannot be well dispersed and remain attached together as aggregates by mutual magnetic attractions. The results here indicate that casein provides the well stabilization of the iron oxide nanoparticles. Casein-MNPs show magnetic property at room temperature, the particles being attracted to the magnet from a homogeneous aqueous dispersion (Fig. S3, Supporting Information). The magnetic properties of casein-MNPs were investigated with a vibrating sample magnetometer (VSM). The saturation magnetization value of casein-MNPs is ~38 emu/g at 25 °C (Fig. S3, Supporting Information), which is lower than that of the bulk magnetite (92 emu/g). The significant softening of magnetic property is caused by the small size of the nanoparticles and the existence of organic coating agents.

The prepared casein-MNPs showed an excellent dispersibility in water, and the obtained suspension was stable for several weeks without noticeable precipitation. This result, at a first instance, gives an evidence of casein modification, because bare MNPs are completely insoluble in water. The modification of casein was confirmed by Fourier transform infrared (FTIR) spectroscopy and TG measurements. Fig. S4 shows the FTIR spectra of bare MNPs (curve a), pure casein (curve b) and casein-MNPs (curve c). The spectra of the casein-MNPs contained a combination of the characteristic bands of the MNPs and the protein. The demonstration of amide I (1641 cm^{-1}) and amide II (1536 cm^{-1}) in the FTIR spectrum of casein-MNPs indicates that casein has been successfully adsorbed on the MNPs. The peak around 576 cm^{-1} corresponds to the characteristic peak from iron oxide particles. TG analysis of casein-MNPs was performed under nitrogen atmosphere to minimize the mass increase due to Fe^{2+} ions' oxidation and only allow the coating agents to decompose thermally. As shown in Fig. S5 (Supporting Information), the TG curve displays two weight loss processes. The first weight loss should be attributed to the dehydration of the samples. The weight loss of 38% from 250 to 650 °C was due to the thermal decomposition of the protein. The existence of coating agents prevents the coalescence of particles due to the steric hindrance and the electrostatic repulsion between the coating proteins.

3.2. Casein-MNPs as peroxidase mimetics and their use in H_2O_2 detection

To investigate the peroxidase-like activity of casein-MNPs, the catalytic oxidation of peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB) in the presence of H_2O_2 was conducted. As shown in Fig. 1, casein-MNPs can catalyze the oxidation of TMB by H_2O_2 to produce the typical blue color reaction very fast. The maximum absorbance of the reaction mixture was 652 nm, which originated from the oxidation of TMB. The control experiments showed that the absorbance of the casein-MNP-TMB-H_2O_2 system was much higher than that of the TMB-H_2O_2 system, and the casein-MNP-TMB system had no absorbance in 652 nm. This indicates that both casein-MNPs and H_2O_2 are needed for the reaction, which is similar to horseradish peroxidase (HRP). For comparison, we studied the catalysis of bare Fe_3O_4 MNPs without modification by protein (curve d). Interestingly, casein-MNPs show much higher activity towards TMB than bare MNPs, which indicates that the protein modification improves the MNP peroxidase-like activity efficiently.

Like HRP, the catalytic activity of the MNPs is dependent on pH and temperature. Thus, the pH and temperature dependent-activity of the casein-MNPs was investigated. The reaction solution pH and temperature dependent response curves are shown in Fig. S6. The results show that the optimal pH and temperature are pH 4.0 and 35 °C, respectively. As shown in Fig. S6, the catalytic oxidation of TMB with H_2O_2 using the casein-MNPs was faster in acidic solutions than that in neutral or basic solutions, which is

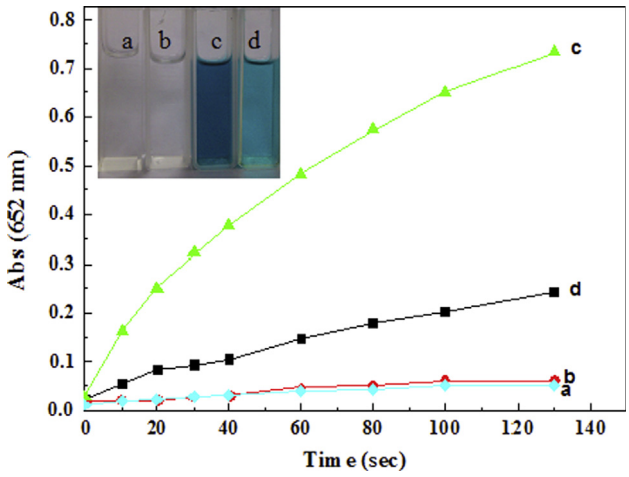

Fig. 1. Time-dependent absorbance changes at 652 nm of TMB in different reaction systems: (a) casein-MNPs+TMB, (b) H_2O_2+TMB, (c) casein-MNPs+H_2O_2+TMB and (d) bare MNPs+H_2O_2+TMB in a pH 4.0 HAc-NaAc buffer (0.1 M) at 35 °C. Inset shows the color change of different samples. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

similar to many other peroxidase-like nanomaterials. Notably, compared with some peroxidase-like nanomaterials, such as $ZnFe_2O_4$ nanoparticles (Su et al., 2012) and BSA-Au cluster (Wang et al., 2011), the catalytic peroxidase-like activity of casein-MNPs can be remained above pH 5.0.

It is important to rule out the possibility that the observed activity is caused by leaching of iron ions into acidic solution. To test this, we incubated casein-MNPs in the standard reaction buffer (pH 4.0) for 1 h and then removed the MNPs from solution with a magnet. We then compared the activity of the leaching solution with that of MNPs under the same conditions. UV–vis spectra indicated the leaching solution had no activity (data not shown), showing that the observed peroxidase-like activity is due to intact casein-MNPs.

To investigate the mechanism of the peroxidase activity of the casein-MNPs, we determined apparent steady-state kinetic parameters for the reaction. Like the nature enzyme catalyzed reaction, a strong dependence was found between initial reaction rate and substrate concentration. Fig. 2 shows the TMB and H_2O_2 concentration-dependent reaction rate. In a certain range of substrate concentration, typical Michaelis–Menten curves were obtained with both TMB and H_2O_2. Michaelis–Menten constant (K_m) and maximum initial velocity (V_{max}) were obtained using Lineweaver–Burk plot (Lineweaver and Burk, 1934, and the results are shown in Table 1. The smaller the value of K_m, the stronger is the affinity between the enzyme and the substrate. The apparent K_m value of the casein-MNPs with H_2O_2 as the substrate (4.75 mM) is much lower than that of bare MNPs (154 mM), and is close to that of HRP (3.70 mM), suggesting that the casein-MNPs have much higher affinity for H_2O_2 than bare MNPs. The apparent K_m value of the casein-MNPs with TMB (0.021 mM) is much lower than that of both bare MNPs (0.098 mM) and HPR (0.434 mM), suggesting that the casein-MNPs have a higher affinity for TMB than both bare MNPs and HRP. Compared with bare MNPs, the presence of casein in casein-MNPs hybrid contributes to the higher affinity of both H_2O_2 and TMB to casein-MNPs.

Yan et al. suggested that ferrous ions may play a dominant role in the catalytic peroxidase-like activity of Fe_3O_4 (Gao and Yan, 2007), and the superficial ferrous ions on MNPs may decompose H_2O_2 into HO free radicals at lower activation energy and play the catalysis role (Liu and Yu, 2011). Therefore, the surface modifications can become a straightforward route to lead to improved

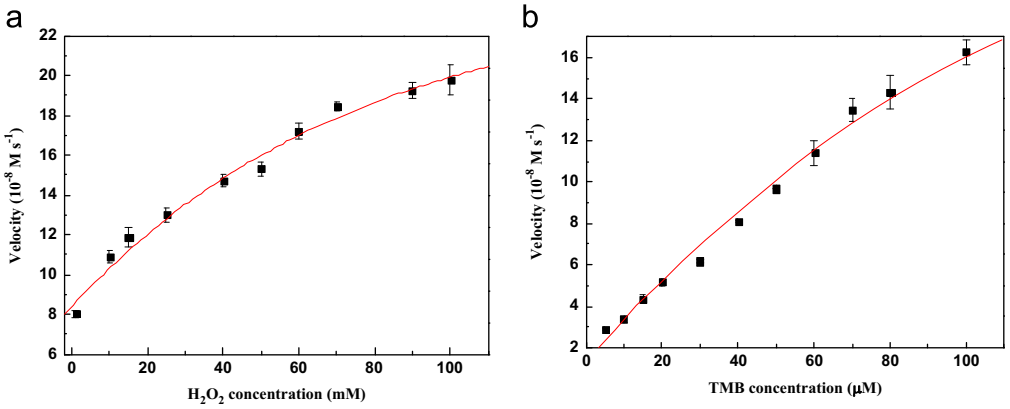

Fig. 2. Correlation of initial reaction velocity with different concentrations of (a) H_2O_2 and (b) TMB. (a) The TMB concentration was 0.08 mM, and (b) the H_2O_2 concentration was 50 mM. Error bars represent the standard deviation based on three repeated measurements.

Table 1
Comparison of the apparent Michaelis-Menton constant (K_m) and maximum reaction rate (V_m).

Catalyst	K_m (mM)		V_m (10^{-8} (M s^{-1})	
	TMB	H_2O_2	TMB	H_2O_2
Casein-Fe_3O_4	0.021	4.75	10.6	15.9
HRP (Gao et al., 2007)	0.434	3.7	10	8.71
Fe_3O_4 (Gao et al., 2007)	0.098	154	3.44	9.78
GO-Fe_3O_4 (Dong et al., 2012)	0.43	0.71	13.08	5.31

catalytic capacities. Any superficial structure that can strengthen the interaction with both TMB and H_2O_2 will favor the affinity toward the two substrates. Casein-MNPs have an apparently improved affinity to H_2O_2 as opposed to bare MNPs. Thus, the introduced casein onto MNPs enhanced the affinity toward H_2O_2 efficiently. For TMB, since both TMB and casein are positively charged, there exists electrostatic repulsion between casein and TMB at pH 4.0. However, due to the amphiphilic property of protein, the superficial casein drags TMB to the vicinity of casein-MNPs via hydrophobic interaction between the hydrophobic chain of protein and the benzenic rings of TMB. As a result, the local TMB concentration is higher than the bulk solution, which favors the oxidation process.

To further investigate the mechanism of the catalysis of casein-MNPs, we measured their activity by varying concentration of TMB at a fixed concentration of H_2O_2 or vice versa. The double reciprocal plots of initial velocity against the concentration of one substrate were obtained over a range of concentrations of the second substrate (Fig. S7, Supporting Information). The slopes of the lines were parallel, which was characteristic of a ping-pong mechanism, as observed for HPR (Gao and Yan, 2007). This indicates that casein-MNPs bind and react with the first substrate and then release the first product before reacting with the second substrate.

Because the catalytic activity of the casein-MNPs is H_2O_2 concentration dependent, the system discussed above could be used to detect H_2O_2. Under the optimal conditions (i.e., 35 °C, 0.1 M pH=4.0 acetate buffer), the method developed was used for H_2O_2 detection. Fig. 3 shows a typical H_2O_2 concentration–response curve where as low as 0.2 μM H_2O_2 can be detected with a linear range from 0.5 μM to 200 μM (Fig. 3b). The detection method based on casein-MNPs gave a much lower detection limit (DL) than that using bare MNPs as catalyst (3.0 μM) (Wei and Wang, 2008).

The stability of the casein-MNPs in wide pH and temperature ranges is crucial to extend their applications. As inorganic materials, casein-MNPs are expected to be more stable than the nature enzyme HRP. To test this, we first incubated casein-MNPs at a range of values of pH (1.5–12) and a range of temperatures (5–90 °C) for 2 h, and then measured its activities under pH 4.0 and 35 °C. Casein-MNPs were indeed found to remain peroxidase activity over the studied pH and temperature range (Fig. S8). In contrast, after treatment at pH lower than 5.0 or temperatures greater than 40 °C for 2 h, the nature enzyme HRP activity dramatically declines. These results showed that the casein-MNPs have strong robustness and can be used for a broad range of applications in the biomedicine and environmental chemistry fields.

3.3. *A simple one-step method for glucose detection using casein-MNP/GO_x nanocomposites*

Since the catalytic activity of casein-MNPs is H_2O_2 concentration dependent and H_2O_2 is the main product of the GO_x-catalyzed reaction, casein-MNPs based sensors can be developed for colorimetric detection of glucose. Many earlier studies have shown that the catalytic activity of enzyme-like nanomaterials is much higher in acidic solution (less than pH 4.0) than that in neutral and basic solutions (Su et al., 2012; Wang et al., 2011). But, the enzyme activity of GO_x will be denatured at acidic pH (less than pH 4.0). Therefore, the detection of glucose by using enzyme-like nanomaterials usually involves two-step analytical processes. First, GO_x was used to catalyze the oxidation of glucose at neutral pH. Then, the H_2O_2 produced served as a substrate for nanomaterials to catalyze the oxidation of the peroxidase substrate to the colored radical at acidic pH. However, the method used in those studies was limited by the fact that it is a multiple-step analysis, and/or non-reusable GO_x.

Intriguingly, the results in the above section have indicated that casein-MNPs can still preserve their 80% activity at pH 5.5 and the optimal pH for glucose oxidase (GO_x) to oxidize glucose is 5.5 (Salloum and Schlenoff, 2004). Furthermore, casein-MNPs display a highly sensitivity for H_2O_2 detection from 0.5 to 200 μM with a DL of 0.2 μM. This may open possibilities for the development of a simple one-step method for turn-on colorimetric sensing of glucose using the nanocomposite of casein-MNP and GO_x if pH 5.5 is chosen. The oxidation of glucose catalyzed by GO_x generates H_2O_2, and the resulting H_2O_2 oxidizes TMB using casein-MNPs as catalyst in the same pH 5.5 buffer solution.

More importantly, casein-MNPs have the potential ability to immobilize the oxidase via the coating proteins. For the oxidase adsorption on casein-MNPs, the driving forces can include the van der Waals force, hydrogen bonding, hydrophobic and electrostatic interaction (Tristán et al., 2009). The isoelectric point (pI) of GO_x and casein is 4.2 and 4.5, respectively. At pH 5.5, where the pH is near to

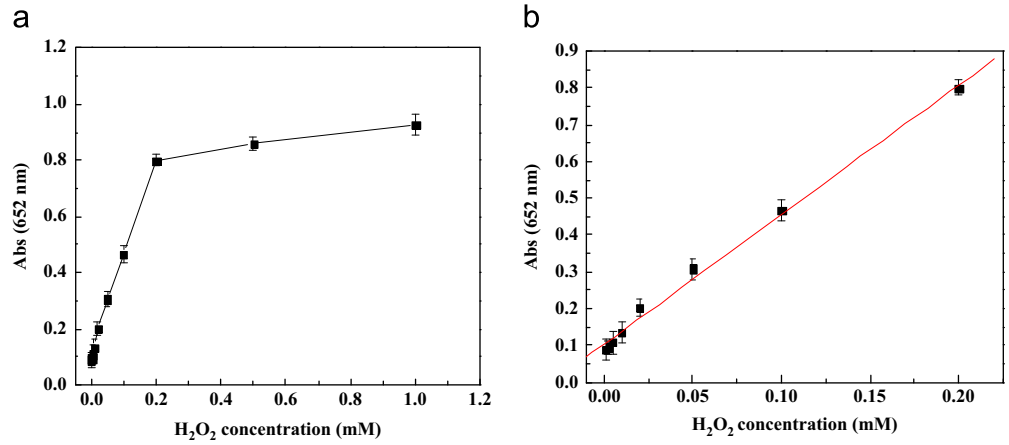

Fig. 3. (a) A dose-response curve for H_2O_2 detection using casein-MNPs. (b) The linear calibration plot for H_2O_2. Error bars represent the standard deviation based on three repeated measurements.

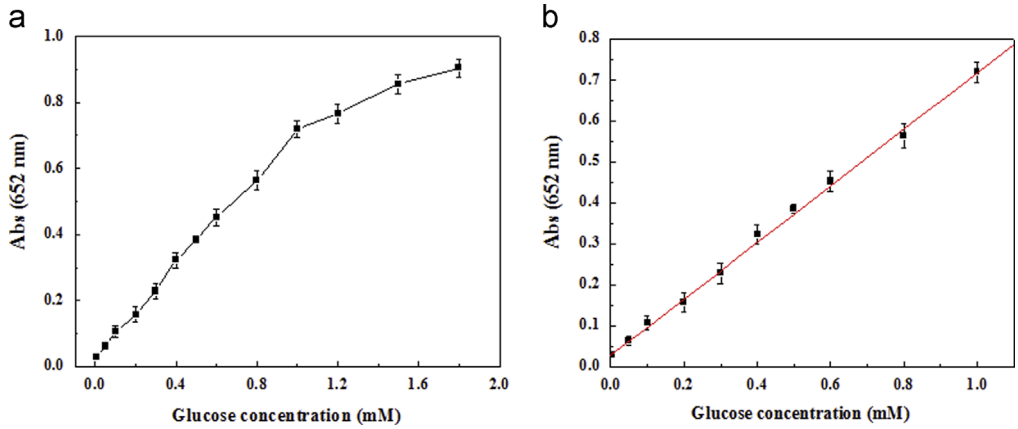

Fig. 4. (a) A dose-response curve for glucose detection using casein-MNP/GO_x nanocomposites. (b) The linear calibration plot for glucose. Error bars represent the standard deviation based on three repeated measurements.

the pI of the two proteins, both casein and GO_x are negatively charged, but the net negative charge of the two proteins is not very high. Clearly, from electrostatic arguments, protein adsorption should be minimized on surfaces with like charge. However, it is generally accepted and confirmed that more hydrophobic surfaces induce greater protein adsorption (Elwing et al., 1987; Prime and Whitesides, 1991; Zhang, et al., 2000). Casein can be thought of as amphiphilic copolymers consisting of blocks with high levels of hydrophobic or hydrophilic amino acid residues. At pH 5.5, where the pH is near to the pI of casein, the less net charge of casein, and the higher hydrophobicity of casein molecules leads to the stronger hydrophobic interaction between casein-MNP and GO_x (Liu and Guo, 2008). This provides an effective immobilization of GO_x on casein-MNPs. Thus, the reusable of GO_x can be performed by the formation of casein-MNPs/GO_x composite. To confirm that GO_x is immobilized on the surface of casein-MNPs, we measured FTIR spectra of casein-MNPs, GO_x, and casein-MNP/GO_x nanocomposites (Fig. S9). Both as proteins, casein and GO_x have similar characteristic bands in the FTIR spectra, but there still exist evident differences in the exact position and intensity of these characteristic bands due to the different structure of casein and GO_x. As shown in Fig. S9, the spectra of the casein-MNP/GO_x nanocomposite contained a combination of the characteristic bands of the casein-MNPs and GO_x. For comparison, we studied the adsorption of GO_x on bare MNPs. It was found that there was almost no GO_x adsorption on bare MNPs, which indicated that the coating casein played important roles in immobilizing GO_x on casein-MNPs.

Therefore, a simple one-step method for turn-on colorimetric sensing of glucose can be achieved by using casein-MNP/GO_x composite. A typical absorption profile for glucose detection using the colorimetric method developed is shown in Fig. 4a. b shows a typical glucose concentration – response curve where as low as 1 μM glucose can be detected with a linear range from 3–1000 μM. The analytical performances comparable to other nano-composite based biosensors reported in references, are shown in Table S1.

Using this method, we detect glucose in human serum sample from a local hospital. Fig. 5 shows the UV – vis spectra and the good colorimetric differentiation. From the UV – vis spectra, it is indicated that the method is largely free from the complicated sample matrix effect of serum sample. According to the calibration curve, the concentration of glucose in serum was 6.6 ± 0.3 mM (118.8 ± 5.4 mg/dL, $n=3$), which agrees well with that measured by the biochemical analyzer in hospital (120.8 mg/dL). These results show that the colorimetric method is very satisfactory in the determination of glucose in serum samples.

To test the selectivity of detection of glucose, the control experiments were taken using fructose, lactose, and maltose. The selectivity of the colorimetric method was shown in Fig. S10. Even when the concentration of control samples was 10 times larger than glucose, the absorbance of glucose was much higher than that of control samples. Thus, the colorimetric method developed here shows high selectivity toward glucose detection.

Glucose determination experiments were repeated for 5 rounds using the same casein-MNP/GO_x nanocomposite to examine the

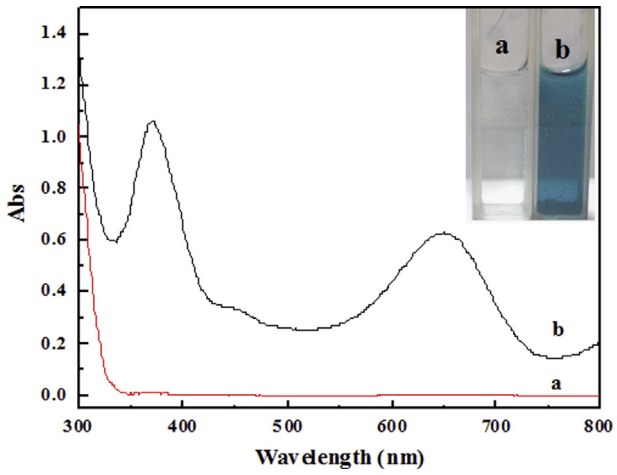

Fig. 5. UV–vis spectra of buffer solution (a) and 10-fold serial dilution of serum samples (b). Inset: Images of production of colored product for buffer solution and serum sample. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

reusability of the nanocomposite. After each experiment the nanocomposite was simply collected by a magnet then regenerated by sonication and washing with deionized water. After 5 rounds of recycle, the nanocomposite still remained above 85% catalytic activity (Fig. S11). It is clear that casein-MNP/GO_x nanocomposite can be reused in the detection of glucose. Due to the low expense of MNP preparation, the characteristic of reusability further enhance the usage of the nanocomposite in practice.

4. Conclusion

The detection of glucose using casein-MNP/GO_x nanocomposites is developed. This method provides several advantages for sensing glucose, including simplicity (one-step detection), reusable enzymes (peroxidase mimic and oxidase), and high sensitivity. Additionally, casein-MNPs possess enhanced peroxidase-like activity when compared to bare MNPs. Thus, the choice of a suitable protein modification is a straightforward and robust strategy to improve the peroxidase-like activity of MNPs. The protein-MNP nanocomposite shows great potential applications in varieties of simple, robust, and easy-to-make analytical approaches in the future.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.09.020.

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