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Novel biosensing platform based on self-assembled supramolecular hydrogel

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ABSTRACT

The supramolecular hydrogel self-assembled from α-cyclodextrin (α-CD) and an amphiphilic triblock copolymer was used for the first time as a biosensing platform by the in-situ incorporation of horseradish peroxidase and polyaniline (PANI) nanoparticles. It was found that the used triblock copolymer could disperse well PANI nanoparticles in aqueous system and then interact with α-CD in the presence of horseradish peroxidase for the formation of supramolecular hydrogel composite. The content of PANI nanoparticles was found to affect the gelation time and gel strength. The circular dichroism analyses showed that the entrapped horseradish peroxidase could retain its native conformation. By electrochemical experiments, the incorporated PANI nanoparticles were confirmed to improve the current response and enzymatic activity, and the fabricated biosensor was found to provide a fast amperometric response to hydrogen peroxide.

1. Introduction

In recent years, the use of hydrophilic hydrogels for the fabrication of biosensors has received much attention [1–8]. It is known that hydrogel matrices can provide relatively stable host environments for various recognition species, including enzymes and electrodes [4,5]. Some examples are polycarbamyl sulphonate hydrogel used to immobilize salicylate hydroxylase, L-lactate dehydrogenase and pyruvate oxidase for the amperometric determination of lactate [6], poly(carboxybetaine) methacrylate hydrogel used to immobilize glucose oxidase for the development of amperometric enzyme glucose sensors [7] and polyacrylamide hydrogel used to entrap tyrosinase for the amperometric determination of phenolic compounds [8]. To obtain these hydrogel matrices, however, potentially toxic additives such as chemical crosslinkers and photo-initiators have been used, which are unfavorable to maintaining the bioactivity of encapsulated enzymes.

Self-assembled supramolecular hydrogels based on the inclusion complex formation of α-cyclodextrin (α-CD) with various polymers are a new kind of hydrogels with many biomedical applications [9–18]. Such hydrogel materials can be formed in mild conditions without the use of potentially toxic photoinitiator or chemical crosslinker, and are particularly suitable for the in-situ encapsulation of some bioactive molecules. For this reason, Li et al. [19] developed injectable drug-delivery systems based on the supramolecular hydrogels formed by poly(ethylene oxide)s and α-CD; Wang et al. [20] obtained the local sustained intramyocardial delivery of erythropoietin within the supramolecular hydrogel based on α-CD and poly(ε-caprolactone)-poly(ethylene glycol) block copolymer; very recently, our group has investigated the supramolecular gelation of a polymeric prodrug for its encapsulation and sustained release by interacting PEGylated indomethacin with α-CD in aqueous system [21]; we have also carried out the in situ entrapment and delivery of plasmid DNA by the supramolecular hydrogel formed from α-CD and cationic block copolymer composed of Pluronic F-68 and poly(L-lysine) segments [22]. Moreover, these supramolecular hydrogels could be used to combine with some nanoparticles such as magnetic iron oxide nanoparticles [23], silver nanoparticles [24] and clay nanoparticles [25] for the development of functional hydrogel nanocomposites. To our knowledge, however, no work has dealt with the use of α-CD-based supramolecular hydrogel for electrochemical biosensing applications.

In this work, we attempt to use α-CD-based supramolecular hydrogel as a biosensing platform by the in-situ incorporation of horseradish peroxidase and polyaniline (PANI) nanoparticles. It is known that horseradish peroxidase is an effective enzyme bioreceptor for the fabrication of enzyme-based sensors [26,27] and PANI nanoparticles have a good electrochemical activity for amperometric biosensing [28]. For this purpose, an amphiphilic triblock copolymer, Pluronic F-127, was first used as the dispersing agent for the preparation of colloidally stable suspension containing PANI nanoparticles and subsequently interacted with α-CD in aqueous system containing horseradish peroxidase for the supramolecular gelation. It was found that the resultant supramolecular hydrogel composite could be used to fabricate an amperometric biosensor with a fast amperometric response to hydrogen peroxide. Moreover, the content of incorporated polyaniline nanoparticles was found to have a great influence on the gelation time, gel strength and cyclic voltammetric behavior.

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2. Experimental

2.1. Materials

Pluronic F-127 (EO_{100}–PO_{65}–EO_{100}) and α-cyclodextrin (α-CD) were purchased from Sigma and used directly. Aniline was purchased from Fuchen Chemical Reagent Factory (Tianjin, China) and used directly. Potassium persulphate ($K_2S_2O_8$) was purchased from Damao chemical factory (Tianjin, China). Horseradish peroxidase (EC1.11.1.7, RZ > 3.0, 250 U/mg) was obtained from Boao Biotechnology Co. Ltd (Shanghai, China). The other chemicals were of analytical grade and used without further purification.

2.2. Preparation and dispersion of PANI nanoparticles

PANI nanoparticles were synthesized according to a reported method [29]. Briefly, 0.10 g aniline was dissolved in 20 mL 1.0 mol/L HCl solution, and then the prepared potassium persulfate solution (0.20 g $K_2S_2O_8$ dissolved in 10 mL 1.0 mol/L HCl) was added drop by drop at ice-bath condition. After stirring for 12 h at 0–5 °C, the mixture was reacted at room temperature for another 12 h. The PANI nanoparticles as dark green powder were collected by filtration, washed with distilled water and dried in vacuum.

To disperse PANI nanoparticles in aqueous Pluronic F-127 solution, various amounts of PANI nanoparticles were added into 10% aqueous Pluronic F-127 solution, and the mixtures were sonicated for 30 min, followed by centrifugation at 4000 rpm for 10 min, resulting in homogeneous dispersions. The content of PANI nanoparticles in aqueous dispersions was determined by thermogravimetric analysis at the temperature range from 50 to 700 °C under nitrogen. By changing the initial amount of PANI nanoparticles, three homogeneous dispersions containing respectively 0.048, 0.144 and 0.548% PANI nanoparticles were obtained and then used to investigate the effects of incorporated PANI nanoparticles.

For the PANI nanoparticles in aqueous dispersions with and without Pluronic F-127, their size and morphology were investigated by a JEM-2010HR transmission electron microscope (TEM, Japan), and their colloidal stability in aqueous medium was evaluated on the basis of the measurement for their optical absorbance with a UV–vis spectrophotometry ((S52, China) set at a wavelength of 550 nm. Square glass cuvettes with a path length of 1 cm were used.

2.3. Supramolecular gelation

For the formation of supramolecular hydrogel composites, aqueous PANI nanoparticle dispersion containing 10% Pluronic F-127 was mixed with an equal volume of 12% α-CD solution containing 5 mg/mL horseradish peroxidase. The mixture system was thoroughly stirred and set aside at ambient temperature. A gelation occurred to result in a physical network incorporated PANI nanoparticle and horseradish peroxidase due to the supramolecular self-assembly between α-CD and the PEO segments of Pluronic F-127. For the characterization of the hydrogel samples, X-ray diffraction measurements were performed by using a Rigaku D/max-2200 type X-ray diffractometer. The radiation source used was Ni-filtered Cu KR radiation with a wavelength of 0.154 nm. The voltage was set to 40 kV, and the current was set to 40 mA. SEM micrographs were taken by a JSM-6330F field emission scanning electron microscope. Before the SEM observation, the hydrogel sample was fixed on aluminum stubs and coated with gold.

To investigate the gelation kinetics, time-sweep rheological analysis was performed by an Advanced Rheometric Extended System with a torsion resolution of 1 nN (ARES, TA Co.) in oscillatory mode with parallel plate geometry (50 mm diameter, 1.0 mm gap) at 25 °C. In this case, the samples were placed on the plate immediately after mixed together and the measurement began two minutes thereafter. To ensure the rheological measurements within a linear viscoelastic region, a dynamic strain sweep was conducted prior to the frequency sweep, and the corresponding strain was determined to be 0.5%. To investigate the mechanical property of the resultant hydrogel, frequency sweep rheological analysis was conducted with the help of the same ARES. In this case, the hydrogel sample was allowed to consolidate for 12 h before beginning the analysis. The frequency applied to hydrogel sample increased from 0.1 to 100 rad/s with a strain of 0.5%. For a comparison study, the supramolecular gelation and gel strength in the absence of PANI nanoparticle were also investigated.

2.4. Circular dichroism analysis

The effect of in-situ entrapment by the supramolecular hydrogel on the conformation of horseradish peroxidase (HRP) was investigated by circular dichroism (CD) analysis. For this purpose, the HRP released from the supramolecular hydrogel composite in 0.01 mol/L phosphate buffer solution (pH 7.4) was collected. For a comparison study, native HRP was also investigated in phosphate buffer solution. The CD spectra were recorded on a J-810 circular dichroism spectrometer (JASCO, Japan) with a 1.0 cm path length rectangular quartz cell controlled by a thermoelectric cell holder. Data were collected from 195 to 260 nm at a scanning rate of 50 nm/min. All spectra were the average of five consecutive scans. Fresh release medium (0.01 mol/L phosphate buffer solution, pH 7.4) was scanned in the same wavelength range for obtaining a baseline to eliminate the background interference.

2.5. Fabrication and characterization of hydrogen peroxide biosensor

The glass carbon electrode (GCE) was used as the base electrode for the biosensor construction. For the purpose of purification, GCE was polished using alumna powder and then washed by the mixed nitric acid/acetone solvents (1:1, v/v) and double distilled water. For the preparation of the amperometric hydrogen peroxide (H_2O_2) biosensor, the GCE was coated by the supramolecular hydrogel composite containing 5 mg/mL horseradish peroxidase and then kept at 4 °C in a refrigerator. For the property measurements of the hydrogen peroxide biosensor, amperometric and cyclic voltammetric experiments were performed under various conditions with a CHI 750 electrochemical workstation (Shanghai Chenghua, China). Cyclic voltammetric experiments were carried out in PBS solutions (0.02 mol/L) containing different concentrations of H_2O_2. The scan range was set as 0 to −1.0 V with a scan rate of 100 mV/s. To optimize the fabrication of the biosensor, the effect of applied potential on the steady-state current of the biosensor was studied in the presence of 1.0 mmol/L H_2O_2 with a potential range of −0.1 to −0.9 V. In steady-state amperometric experiments, a successive addition (10 μL) of 0.20 mol/L H_2O_2 in 0.02 mol/L PBS at an applied potential of −0.7 V under magnetic stirring was carried out. All experiments were carried out with a conventional three-electrode system with the coated GCE as the working electrode and a platinum wire as the auxiliary electrode. For a comparison study, the supramolecular hydrogel without PANI nanoparticles was also investigated in the presence of 5 mg/mL horseradish peroxidase.

3. Results and discussion

3.1. Dispersion of PANI nanoparticles by Pluronic F-127

When PANI nanoparticles were dispersed in 10% aqueous solution of Pluronic F-127 triblock copolymer by ultrasonication, we found that a macroscopically homogeneous dispersion could be obtained. Fig. 1a gives the relative optical absorbance (A/A_0) as a function of the setting time for aqueous dispersions of PANI nanoparticles in the absence and presence of Pluronic F-127. As shown, the A/A_0 value has an obvious decrease in the absence of Pluronic F-127. This may be attributed to the strong hydrophobic interaction among the naked PANI nanoparticles, which makes these particles easier to

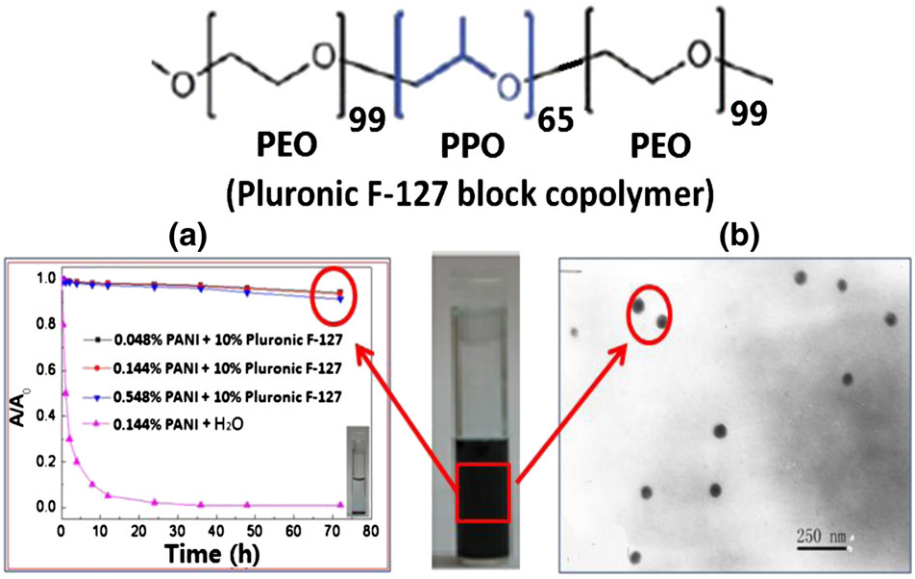

Fig. 1. (a) The relative optical absorbance (A/A_0) as a function of the setting time for aqueous dispersions of PANI nanoparticles in the absence and presence of 10% Pluronic F-127; and (b) the typical TEM image for a macroscopically homogeneous dispersion containing 0.548% PANI nanoparticles and 10% Pluronic F-127.

agglomerate. In the presence of Pluronic F-127, however, the A/A_0 values of three PANI nanoparticle dispersions have only a little change after 3 days, showing their good colloidal stability. In this case, the content of incorporated PANI nanoparticles showed little effects on their dispersibility in the range from 0.048 to 0.548%. Fig. 1b shows the typical TEM image for a macroscopically homogeneous dispersion containing 0.548% PANI nanoparticles and 10% Pluronic F-127. As seen, no aggregates were observed and the dispersed PANI nanoparticles were in the size range of 40–60 nm. These results demonstrate that the used Pluronic F-127 can be used as a good stabilizing agent for the dispersion of PANI nanoparticles. The dispersion of PANI nanoparticles by amphiphilic Pluronic F-127 is based on the noncovalent interaction in water, where hydrophobic PPO blocks adsorb onto the hydrophobic surface of the PANI nanoparticles and hydrophilic PEO blocks dangle into the solvent (water). In this case, the adsorbed Pluronic F-127 chains prevent the aggregation of PANI nanoparticles in aqueous system, resulting in high dispersability of PANI nanoparticles. In fact, Pluronic copolymers have been used as a good stabilizing agent for dispersing single-walled carbon nanotubes [30,31] or graphene sheets [32] in aqueous systems by their selective adsorption, similar to our present study.

3.2. Formation of supramolecular hydrogel nanocomposite

For the resultant colloidal PANI nanoparticle dispersion stabilized by 10% Pluronic F-127, we found that a gelation phenomenon could occur in about 30 min when it was mixed with equal volume of 12% aqueous α-CD solution containing 5 mg/mL HRP, as shown in Fig. 2a. A lower α-CD concentration (smaller than 12%) resulted in a prolonged gelation time more than 60 min, which would be unfavorable for the enzyme encapsulation. In addition, a higher α-CD concentration (greater than 12%) was not used due to limited solubility of α-CD in water although the gelation time may decrease with the increase of α-CD concentration according to our previous study [33].

It is known [34,35] that α-CD could form the crystalline inclusion complexes with poly(ethylene oxide) (PEO) in aqueous solution. Here the inclusion complexes formed by α-CD and the PEO blocks of Pluronic F-127 may be thought to aggregate into the microcrystals, which act as the physical crosslinks and then induce the information of supramolecular polymer networks. To confirm this, we measured the XRD pattern of the freeze-dried hydrogel sample and then compared it to those from α-CD and Pluronic F-127, as shown in Fig. 2b. Pure α-CD is characteristic of multiple diffraction peaks corresponding to its crystalline form, and Pluronic F-127 is characteristic of two main strong peaks at 19.0° and 23.9°. In contrast, the freeze-dried hydrogel sample shows a diffraction pattern quite different from those of α-CD and Pluronic F-127. In particular, a new diffraction peak at $2\theta = 19.8°$ (d = 4.44 Å) was observed for the hydrogel sample. Such a characteristic peak was also found for α-CD/PEO inclusion complexes with the channel-type crystalline structure [34,35]. In addition, the micellization of Pluronic F-127 in aqueous solution may provide another driving force for the gelation. Due to its amphiphilic property, Pluronic F-127 has a critical micelle concentration (*cmc*) of 2.80×10^{-6} mol/L [36], which is far below the concentration of Pluronic F-127 used in this study (10%, w/v). In this case, Pluronic F-127 can self assemble into the polymeric micelles, which may accelerate the formation of hydrogel networks (Fig. 2c).

To investigate the effect of incorporated PANI nanoparticles on the supramolecular gelation, a time sweep measurement for the viscoelastic properties was carried out for each mixed system, in which the storage modulus (G') and loss modulus (G'') were monitored as a function of time, as shown in Fig. 3a. At the initial stage of the measurement, the system remains in a low viscous state and no gelation phenomenon was observed. In this case, the values of G' and G'' were too low to obtain reliable measurements. With passing time, the G' and G'' values of the mixed system were found to have a great change. Particularly, a shift from a predominantly viscous liquid ($G''>G'$) to a strongly viscoelastic solid like material ($G'>G''$) was observed for each mixed system. The corresponding time of the crossover from a viscous behavior to an elastic response could be considered as the gelation time [37]. As a result, the gelation time was determined to be about 42.0 min in the absence of PANI nanoparticles. In contrast, a faster gelation process was found when PANI nanoparticles were incorporated. With the increase of PANI nanoparticles from 0.048 to 0.548%, the gelation time decreased from 32.5 to 10.8 min. This may be attributed to the gathering of PEO blocks around well-dispersed PANI nanoparticles when the PPO blocks of Pluronic F-127 absorbed onto the hydrophobic surfaces of PANI nanoparticles in order to provide good dispersion. In this case, the gathered PEO blocks of Pluronic F-127 would be favorable for the inclusion complexation with α-CD,

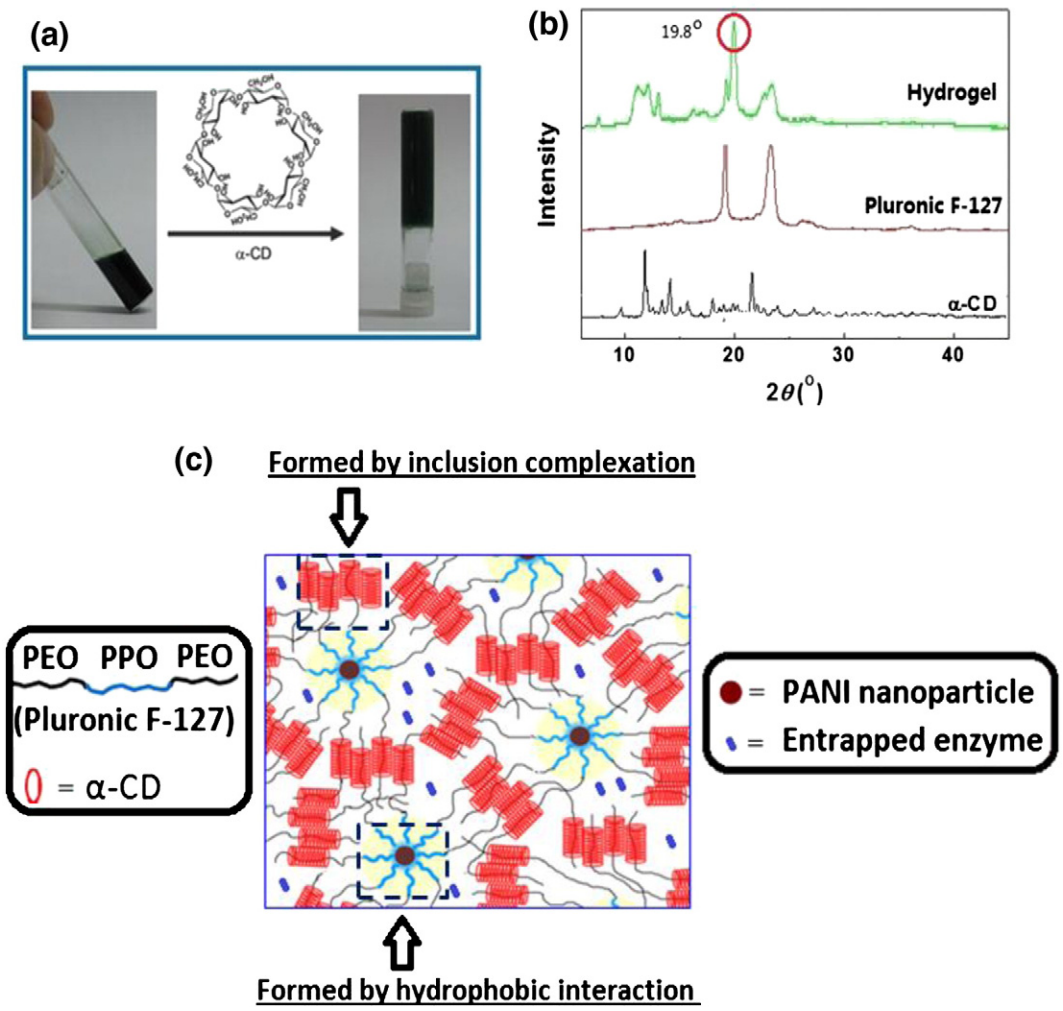

Fig. 2. (a) Photographs for the formation of a supramolecular hydrogel composite when aqueous dispersion containing 0.548% PANI nanoparticles and 10% Pluronic F-127 was mixed with an equal volume of 12% α-CD solution containing 5 mg/mL HRP; (b) X-ray diffraction patterns for α-CD, Pluronic F-127 and freeze-dried supramolecular hydrogel composite shown in Fig. 2a; and (c) the possible structure of the supramolecular hydrogel composite in the presence of PANI nanoparticles.

resulting in a faster gelation process. Similar phenomenon was also observed for the supramolecular hydrogels hybridized with single-walled carbon nanotubes [38].

Further investigation was dealt with the effect of incorporated PANI nanoparticles on the dynamic moduli of resultant supramolecular hydrogel composites. Fig. 3b presents the G' and G'' evolutions of the supramolecular hydrogel without and with PANI nanoparticles as a function of frequency. Regardless of incorporated PANI nanoparticles, the G' values of these hydrogels exhibit a substantial elastic response, which are greater than the loss modulus G'' over the entire range of frequency, indicating the formation of strong and rigid hydrogels. However, the incorporation of PANI nanoparticles resulted in the decreases of storage modulus and loss modulus, in particular in the case of 0.548% PANI nanoparticles. Such an effect may be explained from the mechanism of hydrogel formation and PANI nanoparticle dispersion by Pluronic F-127. Due to the hydrophobic interaction between Pluronic F-127 and PANI nanoparticle, the micellization of Pluronic F-127 in aqueous solution was weakened in the presence of PANI nanoparticle, which would result in the decrease of cross-linking points in the formed supramolecular hydrogel. Similar experimental result was also found for the supramolecular hydrogels hybridized with single-walled carbon nanotubes [38]. Fig. 4 shows the SEM images for the morphologies of the supramolecular hydrogels without and with PANI nanoparticles. Different from the native supramolecular hydrogel, the supramolecular hydrogel with PANI nanoparticles were observed to have a relatively loose structure with plentiful undulant pimples.

3.3. Conformation of in-situ entrapped horseradish peroxidase

For the resultant supramolecular hydrogel, its suitability for the in situ encapsulation of HRP as the enzyme bioreceptor was investigated by circular dichroism (CD) analysis. Fig. 5 shows the CD spectra of native HRP and entrapped HRP. Native HRP exhibited a negative minimum in the ultraviolet region at about 208 nm, which is characteristic of α-helical structure of enzyme protein [39]. After the entrapment, the HRP is also characteristic of a negative minimum at about 208 nm, which shows insignificant difference from native HRP. These facts indicate that the entrapped HRP could keep its original structure and the entrapment does not affect the enzyme protein structure to any significant extent.

3.4. Properties of fabricated biosensor

To construct the amperometric biosensor for hydrogen peroxide, the resultant supramolecular hydrogel composite was used, and the electrocatalytic behavior of entrapped HRP was evaluated by cyclic voltammetry. Fig. 6 shows the cyclic voltammograms of the modified electrode in pH 7.0 phosphate buffer saline (PBS) at a scan rate of

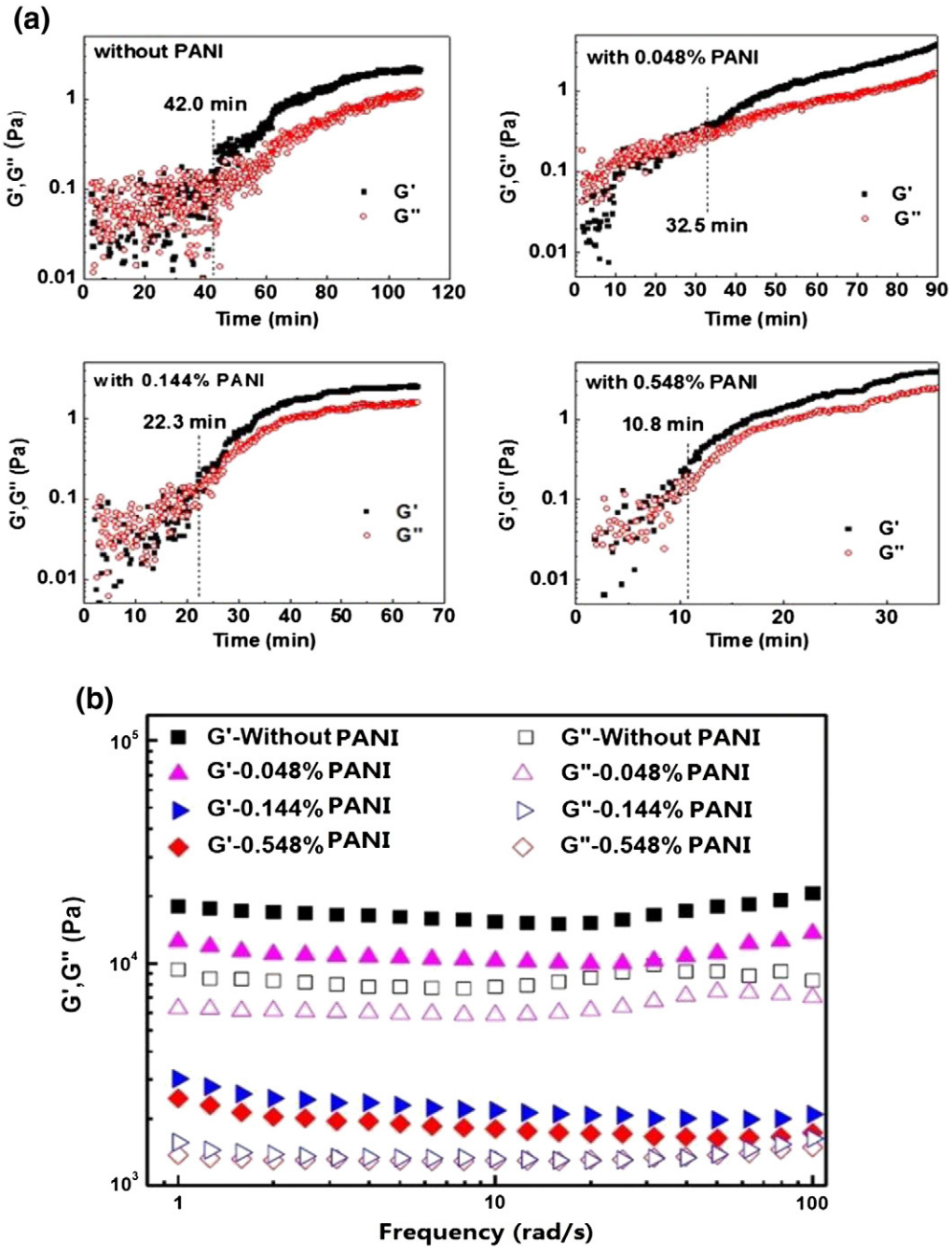

Fig. 3. Effects of PANI nanoparticles on the gelation kinetics (a) and dynamic moduli (b) of resultant supramolecular hydrogel composites. Fixed conditions: α-CD, 6.0% (in the mixed system); Pluronic F-127, 5.0% (in the mixed system); and HRP, 5 mg/mL. Test conditions: 0.5% strain and 25 °C.

100 mV/s. In the absence of H_2O_2, the enzyme electrode gave no response, and only a background current was observed. After H_2O_2 was added, an electrocatalytic characteristic was observed with the increase of reduction current, particularly in the case of higher H_2O_2 amount (2.0 mM). For our tests, the modified glassy carbon electrode was immersed in PBS (pH 7.4) to remove the loosely adsorbed layers and was stored at 4 °C in a refrigerator under dry conditions when not in use. Its cyclic voltammetric responses in PBS (pH 7.4) show no obvious changes after several cycles. For the investigation of its storage stability, the cathodic peak current was measured using the same electrode and was found to have only 18% decrease of its initial response after 14 days.

To optimize the fabrication of the biosensor, the effect of applied potential on the steady-state current of the biosensor was studied in the presence of 1.0 mmol/L H_2O_2. From Fig. 7, it was found that the electrode response to H_2O_2 increased with the change of applied potential from −0.4 to −0.7 V. Beyond −0.7 V, a very little increase of the current response was found when the applied potential increased. Considering the negative effect of the background response, the working potential for this biosensor was fixed to be −0.7 V.

Fig. 8a shows the amperometric responses of the biosensors fabricated from the supramolecular hydrogel composites without and with PANI nanoparticles to the successive addition of 0.20 mol/L H_2O_2 in 0.02 mol/L PBS at an applied potential of −0.7 V. As observed,

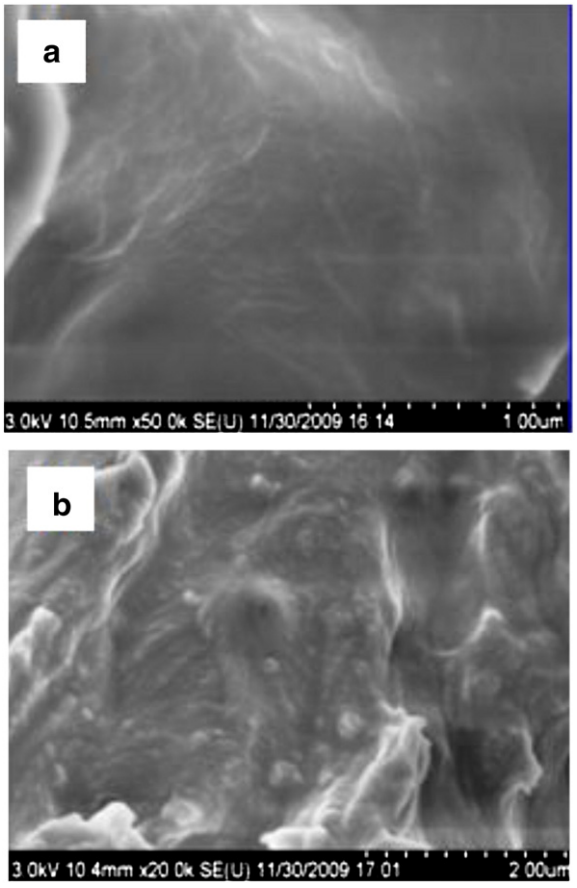

Fig. 4. SEM images for the morphologies of the supramolecular hydrogels without (a) and with (b) PANI nanoparticles.

these fabricated biosensors responded to the substrate so rapidly that it could obtain about 95% of the steady-state current within 6 s. In particular, the current response increased with the increase of PANI nanoparticle content, indicating that the incorporated PANI nanoparticles could enhance the biosensor sensitivity. In fact, PANI has been widely used in the biosensor architecture due to its characteristics of easy preparation, high environmental stability and good electro-conductivity [40–45]. They can provide not only a suitable environment for the immobilization of biomolecules and improve impressive signal amplification [41–43], but also can act as a mediator for enzyme electrodes which

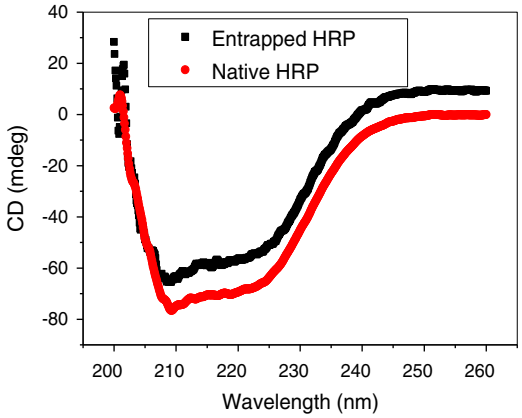

Fig. 5. Circular dichroism spectra of native HRP and the HRP encapsulated in the supramolecular hydrogel (phosphate buffer; pH 7.4; 25.0 °C). Composition of used hydrogel composite: α-CD, 6.0% (in the mixed system); Pluronic F-127, 5.0% (in the mixed system); PANI nanoparticles, 0.144%; and HRP, 5 mg/mL).

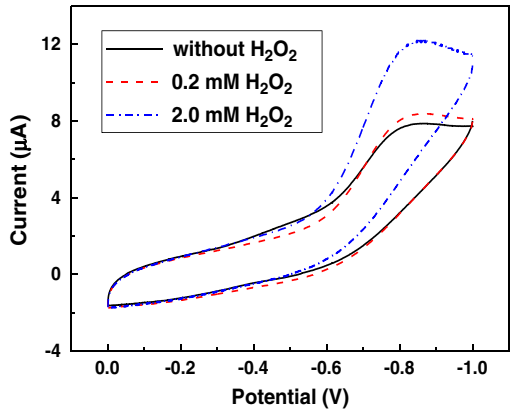

Fig. 6. Cyclic voltammetric responses of the modified electrodes in 0.02 mol/L PBS with and without H_2O_2 (scan rate is 100 mV/s). Composition of used hydrogel composite: α-CD, 6.0% (in the mixed system); Pluronic F-127, 5.0% (in the mixed system); PANI nanoparticles, 0.144%; and HRP, 5 mg/mL).

could make couples electrons directly from the enzyme active site to the electrode surface [40,44,45].

Fig. 8b gives the calibration plots between the current and the concentration of H_2O_2 for the fabricated biosensors. As seen, these biosensors had a linear range of H_2O_2 concentration from 0.2 mmol/L to 5.0 mmol/L. The apparent Michaelis–Menten constant (K_m^{app}), which gives an indication of the enzyme–substrate kinetics, could be estimated from the electrochemical version of the Lineweaver–Burk equation [46]:

$$1/I_{ss} = 1/I_{max} + K_m^{app}/(I_{max}c) \quad (1)$$

where I_{ss} is the steady-state current after the addition of substrate, c is the bulk concentration of the substrate and I_{max} is the maximum current under saturated substrate conditions. In the case of supramolecular hydrogel composite without PANI nanoparticles, the K_m^{app} and I_{max} were found to be 5.19 mmol/L and −15.9 μA, respectively, with a determination coefficient of 0.997. In the case of supramolecular hydrogel composite with 0.048% PANI nanoparticles, the K_m^{app} and I_{max} were found to be 3.98 mmol/L and −16.2 μA, respectively, with a determination coefficient of 0.994. In the case of supramolecular hydrogel composite with 0.144% PANI nanoparticles, the K_m^{app} and I_{max} were found to be 4.07 mmol/L and −18.5 μA, respectively, with a determination coefficient of 0.986. In the case of supramolecular hydrogel composite with 0.548% PANI nanoparticles, the K_m^{app} and I_{max} were found to be 4.42 mmol/L and −31.2 μA, respectively, with a determination coefficient of 0.994. Compared to the K_m^{app} value in the absence of PANI

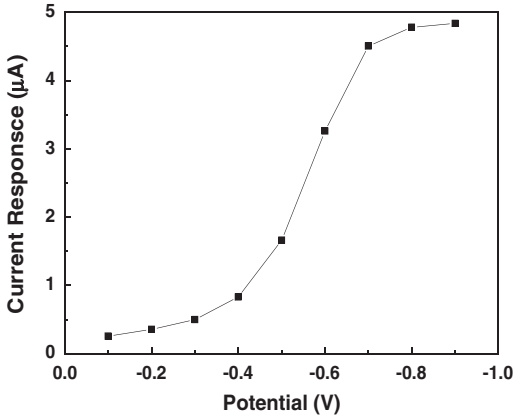

Fig. 7. Effect of the potential on the peak current of the electrocatalytic H_2O_2 oxidation on the modified electrode in the presence of 1 mmol/L H_2O_2 in 0.02 mol/L PBS. Composition of used hydrogel composite: α-CD, 6.0% (in the mixed system); Pluronic F-127, 5.0% (in the mixed system); PANI nanoparticles, 0.144%; and HRP, 5 mg/mL).

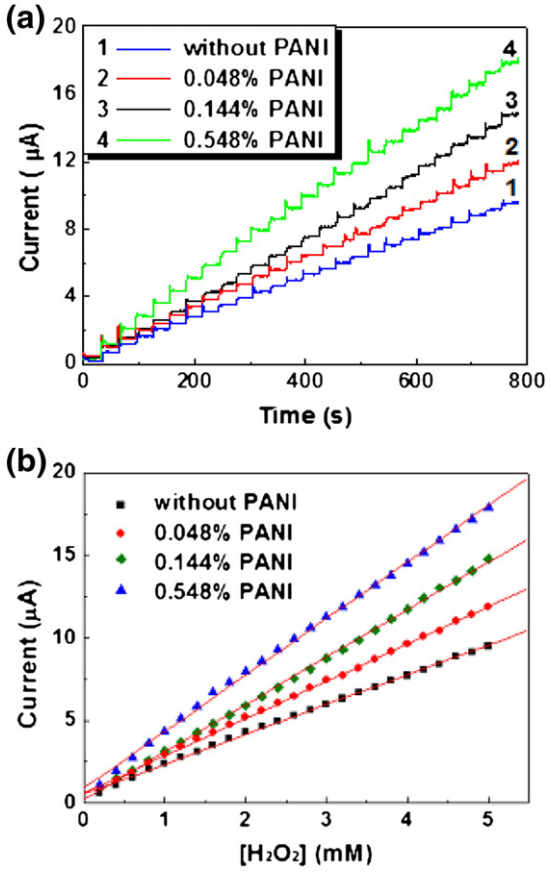

Fig. 8. (a) Amperometric responses of the electrodes modified by the supramolecular hydrogel composites without and with PANI nanoparticles to the successive addition of 0.20 mol/L H_2O_2 in 0.02 mol/L PBS at an applied potential of −0.7 V; and (b) calibration plots between the current and the concentration of H_2O_2 for the electrodes modified by the supramolecular hydrogel composites without and with PANI nanoparticles.

nanoparticles, all of the K_m^{app} values in the presence of PANI nanoparticles were found to have an obvious decrease. The smaller K_m^{app} value means higher enzymatic activity [46]. It seems that the incorporation of PANI nanoparticles could improve the enzymatic activity of the fabricated biosensor.

4. Conclusions

The self-assembled supramolecular hydrogel based on α-CD and Pluronic F-127 was investigated for the first time as a biosensing platform. By combining dual roles of Pluronic F-127 in dispersing PANI nanoparticles and forming inclusion complexes with α-CD, horseradish peroxidase (HRP) as the enzyme bioreceptor and PANI nanoparticles as the property enhancer could be incorporated simultaneously into the resultant supramolecular hydrogel matrix. The entrapped HRP could retain its native conformation due to mild gelation conditions. The incorporated PANI nanoparticles had a great influence on the gelation time, gel strength and cyclic voltammetric behavior. By using the resultant supramolecular hydrogel composite, the fabricated biosensor was found to provide a fast amperometric response to hydrogen peroxide.

Acknowledgments

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