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Hemin-functionalized WS$_2$ nanosheets as highly active peroxidase mimetic for label-free colorimetric detections of H$_2$O$_2$ and glucose was reported.
Hemin-functionalized WS$_2$ nanosheets as highly active peroxidase mimetic for label-free colorimetric detections of H$_2$O$_2$ and glucose

Qiao Chen$^{ab}$, Jia Chen$^a$, Cunji Gao$^a$, Mingliang Zhang$^a$, Junying Chen$^b$ and Hongdeng Qiu$^a$

Hemin-functionalized WS$_2$ nanosheets (hemin/WS$_2$-NSs) was firstly obtained by the hemin assembled on the surface of few-layered WS$_2$ nanosheets (WS$_2$-NSs) via van der Waals interactions. Significantly, this new material possessed the advantages of both hemin and WS$_2$ nanosheets and exhibited some unique properties. Firstly, Hemin/WS$_2$-NSs had intrinsic peroxidase-like activity, which could effectively catalyze oxidation of the substrate 3,3',5,5'-tetramethylbenzidine (TMB) by H$_2$O$_2$ to produce a typical blue color reaction. Secondly, the activity of hemin/WS$_2$-NSs was much higher than the activity of hemin or WS$_2$-NSs alone. The catalytic activity followed the typical Michaelis–Menten kinetics and was dependent on temperature, pH, H$_2$O$_2$ concentration, as well as reaction time. Based on this finding, a new highly sensitive and selective colorimetric method for H$_2$O$_2$ and glucose detection was developed. This method was simple, inexpensive for glucose detection using glucose oxidase (GOx) and hemin/WS$_2$-NSs with a linear range from $0.5 \times 10^{-5}$ to $2.0 \times 10^{-4}$ mol L$^{-1}$ with a detection limit of $1.5 \times 10^{-6}$ mol L$^{-1}$. The good catalytic activity and low-cost make the hemin/WS$_2$-NSs a useful biocatalyst for a wide range of potential applications in environmental chemistry, biotechnology and clinical diagnostic.

1. Introduction

Colorimetric methods are particularly attractive for point-of-use applications because they can be visually and simply observed by the naked eye.$^{1,2}$ Colorimetric biosensing does not require expensive or sophisticated instrumentation and can be applied to field analysis and point-of-care diagnosis.$^{3-6}$ With the advent of nanotechnology, Enzyme-mimetic nanomaterials have emerged as a new class of ideal and important tools for colorimetric detection.$^{7,8}$ Nanomaterials represent promising candidates for artificial peroxidase mimetics possess significant advantages over natural enzymes (such as horseradish peroxidase, HRP) including high stability, low-cost of preparation, simple storage, flexibility in composition and structure design, tunable catalytic activities.$^9$ To this end, the artificial peroxidase mimetics with good catalytic properties will possess a brilliant future in enzyme-related applications. The Fe$_3$O$_4$ magnetic nanoparticles (MNPs) was first reported that possessed intrinsic peroxidase-like activity,$^{10}$ and some other metal oxide nanomaterials were also found to possess intrinsic peroxidase-like activity, such as MoS$_2$ nanosheets,$^{11}$ ZnFe$_2$O$_4$ MNPs,$^{12}$ Co$_3$O$_4$ NPs,$^{13}$ and V$_2$O$_5$ nanowires.$^{14}$ Furthermore, carbon nanomaterials such as single-wall,$^{15}$ helical carbon nanotubes,$^{16}$ graphene oxide,$^{17}$ carbon nanodots,$^{18}$ and multicolor luminescent carbon nanoparticles$^{19}$ have also been proved to possess intrinsic peroxidase catalytic activity. So far, all these enzyme-like nanomaterials have already found wide applications in a variety of fields, including biosensing,$^{20,21}$ immunoassays,$^{22}$ cancer diagnostics and therapy, neuroprotection, stem cell growth, pollutant removal,$^7$ and so on.

Tungsten disulfide (WS$_2$) transitions from indirect-gap semiconductor with a gap of 1.3 eV in bulk to direct-gap in a monolayer, the indirect-to-direct gap transition results in strong enhancement of photoluminescence efficiency.$^{22,23}$ So far, WS$_2$ nanosheets have received considerable attention in hydrogen evolution reactions,$^{24,25}$ energy storage,$^{26}$ electronic devices,$^{27}$ biomedicine,$^{28}$ biosensors$^{29,30}$ et al. Lin’s group$^{31}$ recently reported that WS$_2$ nanosheets possessed intrinsic peroxidase-like catalytic activity. The WS$_2$ nanosheets could catalyze the oxidation of TMB by H$_2$O$_2$ to produce the typical blue color reaction. On the basis of this finding, colorimetric methods for the detection of H$_2$O$_2$ and glucose were developed. Hemin (iron protoporphyrin) is the active center of the heme-protein family including cytochromes, peroxidases, myoglobins, and hemoglobins, which has the peroxidase-like activity similar to the peroxidase enzyme.$^{32}$ It also possesses a large extinction coefficient in the visible-light region, predictable rigid structures, and prospective photochemical electron-transfer ability. However, the catalytic activity of hemin was limited because of its oxidative self-degradation and molecular aggregation to inactive dimers in aqueous solution. A way to solve this problem
is immobilizing hemin on high surface area materials. As we know that the deposition of porphyrin on the graphene oxide sheets through π–π interaction was successfully realized. Based on the above works, we thought that it might be possible for WS$_2$ nanosheets to serve as an available platform because of its similar 2D structure with graphene, which could be used to form composite nanomaterials with some plane-like molecules. If the Hemin-functionalized WS$_2$ nanosheets (hemin/WS$_2$-NSs) could be obtained, it will provide excellent opportunities for applications in the field of artificial enzyme mimetic for biosensor and bioanalysis. In this paper, hemin/WS$_2$-NSs were synthesized by simple wet-chemical strategy via van der Waals interactions. Significantly, the new nanocomposite possessed both excellent properties of WS$_2$ and hemin. It was found that WS$_2$ not only could effectively load hemin, but also provided a beneficial microenvironment for keeping the high activity of hemin. The activity of hemin/WS$_2$-NSs was much higher than the activity of hemin alone. Based on this finding, a new label-free colorimetric sensor for H$_2$O$_2$ and glucose was successfully developed (Scheme 1). The developed method exhibited good sensitivity and selectivity.

Scheme 1. Schematic illustration of colorimetric detection of glucose by using glucose oxidase (GOx) and hemin/WS$_2$-NSs-catalyzed reactions.

2. Experimental section

2.1. Materials and Instrumentation

Tungsten disulfide (WS$_2$) nanosheets (26 µg/mL, 1–4 monolayers, >99% Purity in dry phase) were purchased from XF NANO Materials Tech Co., Ltd (Nanjing, China). Hemin, o-phenylenediamine (OPD), 3,3',5,5'-tetramethylbenzidine (TMB) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) dianion salt (ABTS) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Glucose oxidase, glucose, fructose, maltose and lactose were obtained from Sangon Biological Engineering Technology & Services Co., Ltd (Shanghai, China). H$_2$O$_2$ (30%), NaCH$_3$COOH (NaAc), and CH$_3$COOH (HAc) were purchased from Guangfu Chemical Reagent Factory (Tianjin, China). All other chemicals were of analytical grade and used without further purification. Water was purified with a MilliGQ plus 185 equip from Millipore (Bedford, MA, USA) and used throughout all experiments. UV–visible absorption spectra and kinetic measurements were carried out on a Lambda 35 UV–vis spectrophotometer (Perkin-Elmer, USA). Transmission electron microscopy (TEM) images, high-resolution transmission electron microscopy (HRTEM) images and selected are a electron diffraction (SAED) images were performed on a Tecnai G2 TF20 transmission electron microscope (FEI, USA). Scanning electron microscopy (SEM) images were recorded on a JSM–6701F field emission scanning electron microscope (Japan). X-ray diffraction (XRD) patterns were carried out in an X’Pert PRO X-ray diffractometer (PANanalytical, Netherlands). X-ray photoelectron spectra (XPS) analyses were conducted using an ESCALAB 250Xi X-ray photoelectron spectroscopy (ThermoFisher Scientific, USA).

2.2. Preparation of hemin-functionalized WS$_2$ nanosheets

Hemin-functionalized WS$_2$ nanosheets (hemin/WS$_2$-NSs) were prepared based on a previously reported method with minor modifications. Briefly, 50 mL of 26 µg/mL hemin was added to 50 mL of the homogeneous WS$_2$ nanosheets (26 µg/mL) solution, followed by incubating for 4 h at 60 °C. After incubation, the resulting solution was then centrifuged at 16000 rpm for 30 min and black sediments, hemin/WS$_2$-NSs, were obtained. Furthermore, the as-prepared hemin/WS$_2$-NSs were washed by repeated centrifugation (16000 rpm, 30 min) and resuspension in ethanol for three times. And then, the sediments were resuspended in water to prepare the hemin/WS$_2$-NSs solution under sonication.

2.3. Detection of H$_2$O$_2$ and glucose

H$_2$O$_2$ detection was performed as follows: hemin/WS$_2$-NSs (100 µL, 32 µg/mL) TMB (100 µL, 10 mM) and 600 µL HAc-NaAc buffer (0.2 mM, pH 4.0) were added into a 1.5 mL polypropylene tube. 200 µL of H$_2$O$_2$ with different concentration was added. Subsequently, the mixed solutions were incubated for 30 min at 40 °C. Finally, 20 µL H$_2$SO$_4$ (20%, v/v) was added into the above solution mixture to stop the catalytic reaction, and the absorbance of the resultant mixture at 450 nm was used for quantification of H$_2$O$_2$. All experiments were repeated three times and each sample solution was measured five times.

Glucose detection was realized as follows: A 20 µL volume of 10 mg/mL GOx solution was added into a 1.5 mL polypropylene tube, followed by the addition of 280 µL HAc-NaAc buffer (0.2 mM, pH 4.0) containing different concentrations of glucose, the resulting solution was maintained at 37 °C for 30 min to produce H$_2$O$_2$. And then, 100 µL hemin/WS$_2$-NSs (32 µg/mL), 100 µL TMB (10 mM) and 500 µL HAc-NaAc buffer (0.2 mM, pH 4.0) were successively injected into the resulting solution, and allowed to further incubate for 30 min at 40 °C. Finally, 20 µL H$_2$SO$_4$ (20%, v/v) was added into the above reaction mixture to stop the catalytic reaction, and the absorbance of the resultant mixture at 450 nm was used for quantification of glucose. All experiments were repeated three times and each sample solution was measured five times.

3. Results and discussion

3.1. Characterization of WS$_2$ nanosheets

Fig. S1(A) showed a TEM image of layered WS$_2$ nanosheets. The SAED pattern of WS$_2$ nanosheets as an inset image in Fig. S1(B) reveals a crystal structure and diffraction points of (101) and (110) planes. The HRTEM image (Fig. S1(C), (D)) clearly illustrated the periodic atomic arrangement of the WS$_2$ nanosheets with a layer separation of about 2.64 Å assigned to the WS$_2$ (101) plane.

3.2. Formation of hemin-functionalized WS$_2$ nanosheets

UV–vis absorption spectra were used to characterize the formation of hemin-functionalized WS$_2$ nanosheets. As shown in Fig. 1, the WS$_2$-NSs dispersion displays a characteristic peak at around 241 nm and 625 nm, which was consistent with previous reports (Fig. 1 curve a). The spectrum of hemin solution shows a strong peak at around 400 nm attributed to the Soret band, as well as a group of weak peaks between 500 and 700 nm ascribed to the Q-bands (Fig. 1 curve b) After incubation, the resulting solution was then centrifuged at 16000 rpm for 30 min and black sediments, hemin/WS$_2$-NSs, were obtained. Furthermore, the as-prepared hemin/WS$_2$-NSs were washed by repeated centrifugation (16000 rpm, 30 min) and resuspension in ethanol for three times. And then, the sediments were resuspended in water to prepare the hemin/WS$_2$-NSs solution under sonication.
curve c). After reduction, the color of the WS$_2$-NSs dispersion changes from pale-yellow (Fig. 1, inset a) to brown (Fig. 1, inset b). The hemin/WS$_2$-NSs aqueous dispersion exhibit a broad absorption at 254 nm, which corresponds to the characteristic peaks of WS$_2$-NSs with a large bathochromic shift and a broad absorption peak was observed at around 400 nm, close to the Soret band of hemin. It is clearly demonstrated that WS$_2$-NSs adsorbed hemin molecules at the surface of nanosheets through van der Waals interactions, which allowed them to be well dispersed in ethanol solution, where a characteristic absorption of hemin was observed from complex nanosheets. Particularly, the dispersion is stable and no obvious precipitates are observed after being stored for more than 3 months.

3.2. Peroxidase-like activity of hemin/WS$_2$-NSs

It is well known that peroxidase can catalyze the oxidation of a peroxidase substrate to produce a color change. To assess the peroxidase-like activity of the hemin/WS$_2$-NSs, TMB–H$_2$O$_2$ reaction was used as a model reaction system. The peroxidase-like activity of hemin/WS$_2$-NSs was first evaluated by the catalytic oxidation of peroxidase substrate TMB in the presence of H$_2$O$_2$ to produce the typical blue color reaction (Fig. S3) with maximum absorbance at 369 nm and 652 nm, increased with the increasing of reaction time (Fig. 3A), which originates from the one-electron oxidation of TMB. However, only addition of H$_2$O$_2$ or hemin/WS$_2$-NSs to TMB solution the absorbance at 652 nm displayed negligible change (Fig. 3B), indicating no oxidation reaction occurred. These results clearly indicated hemin/WS$_2$-NSs exhibited intrinsic peroxidase-like activity and not only H$_2$O$_2$ but hemin/WS$_2$-NSs were required for the reaction, as was also the case for horseradish peroxidase (HRP). Hemin is the active site in peroxidase enzyme, and exhibits the catalytic activity similar to the peroxidase enzyme. It is also reported that WS$_2$-NSs possess intrinsic peroxidase-like activity. As shown Fig. 4. For a comparison study, the response of TMB–H$_2$O$_2$ system was also investigated in the presence of hemin alone or WS$_2$-NSs alone. The results showed that the hemin/WS$_2$-NSs exhibited much higher activity than hemin itself or WS$_2$-NSs alone. To further investigate the catalytic activity of hemin/WS$_2$-NSs, we compared the catalytic activity with hemin and WS$_2$-NSs at the different concentrations of H$_2$O$_2$ (Fig. S4). We could see that the hemin/WS$_2$-NSs exhibited much higher activity than hemin itself or WS$_2$-NSs alone. So, the synergetic effects between hemin and WS$_2$-NSs would result in the high catalytic activity of hemin/WS$_2$-NSs. The peroxidase-like activity of hemin/WS$_2$-NSs was further confirmed by the catalytic oxidation of other peroxidase substrates such as ABTS and OPD in the presence of H$_2$O$_2$. The results are shown in Fig. S5. It can be seen from Fig. S5 that hemin/WS$_2$-NSs could catalyze ABTS to give a green color and catalyze OPD to give an orange color.

![Image](https://via.placeholder.com/150)

Fig. 1 UV-visible spectra of WS$_2$-NSs dispersion (a), hemin/WS$_2$-NSs suspension (b), hemin solution (c). Inset: photographs of WS$_2$-NSs (a), hemin/WS$_2$-NSs (b), hemin solution (c).

The SEM images of the as-prepared WS$_2$-NSs and hemin/WS$_2$-NSs are shown in Fig. S2(A) and (B), respectively, which were used to confirm the nanosheet structure of hemin/WS$_2$-NSs, indicating that hemin molecules adsorbed on the surface of WS$_2$-NSs would not break their original nanosheet morphology. To confirm the chemical composition, the XPS survey of WS$_2$-NSs and as-prepared hemin/WS$_2$-NSs were clearly illustrated in Fig. 2 (A). As seen in this figure, the relative intensities of C 1s and O 1s binding energy at hemin/WS$_2$-NSs increase significantly when compared to that of WS$_2$-NSs. Meanwhile, a weak peak related to Fe 2p binding energy appears, indicating the formation of hemin/WS$_2$-NSs. X-ray diffraction (XRD) spectra was employed to further explore the crystal structures of WS$_2$-NSs and hemin/WS$_2$-NSs. The results obtained are presented in Fig. 2 (B). It can be seen that both WS$_2$-NSs and hemin/WS$_2$-NSs are mainly identified as 2H WS$_2$, which have a dominant peak appearing at 14.36°, representing the (002) plane (PDF card no. 8724-17).

In order to further improve the peroxidase-like activity of hemin/WS$_2$-NSs, substrates TMB and H$_2$O$_2$ was used as a proof-of-principle reaction system. As expected, like HRP and NPs-based peroxidase mimetics, the catalytic activity of hemin/WS$_2$-NSs is also dependent on temperature, pH, and H$_2$O$_2$ concentration. The catalytic activity was increased with the increasing of catalyst concentration and reaction time. The catalytic activity was increased with the reaction time and slowed down after 30 min. It was reported that the catalytic activity of HRP was largely inhibited after.
incubation at pH lower than 4.0 or temperature higher than 50 °C. In contrast, hemin/WS\textsubscript{2}-NSs exhibited high catalytic activity over a wide range of pH (2.0–7.0) and temperature (30–60 °C), the robustness of hemin/WS\textsubscript{2}-NSs make them potentially applicable under harsh conditions (Fig. S6). Like HRP and other nanomaterial-based peroxidase mimics, the catalytic activity of hemin/WS\textsubscript{2}-NSs was inhibited at high H\textsubscript{2}O\textsubscript{2} concentration. Under our experimental conditions, the optimal temperature, reaction time, H\textsubscript{2}O\textsubscript{2} concentration, and pH are 40 °C, 30 min, 150 mmol L\textsuperscript{-1}, and 4.0, respectively.

3.3. Kinetic analysis

To investigate the catalytic mechanism of the peroxidase-like activity of the hemin/WS\textsubscript{2}-NSs, apparent steady-state kinetic parameters for the peroxidase-like color changing reaction were determined by changing the concentrations of TMB or H\textsubscript{2}O\textsubscript{2} in this system while keeping the other one constant. Kinetic experiments were carried out using 100 μL of hemin/WS\textsubscript{2}-NSs in 1.0 mL of NaAc–HAc buffer solution (0.2 mM, pH 4.0) at 40 °C. The error bars represent the standard error derived from three repeated measurements. (A) The concentration of TMB was varied. (B) The concentration of H\textsubscript{2}O\textsubscript{2} was 0.12 mmol L\textsuperscript{-1}, and the H\textsubscript{2}O\textsubscript{2} concentration was varied. (C, D) Double reciprocal plots of activity of hemin/WS\textsubscript{2}-NSs with the concentration of one substrate (TMB or H\textsubscript{2}O\textsubscript{2}) fixed and the other varied.

3.4. Detection of H\textsubscript{2}O\textsubscript{2} and glucose

On the basis of the above optimum assay conditions, we evaluated the sensitivity of the proposed label-free colorimetric method for the detection of H\textsubscript{2}O\textsubscript{2} and glucose. Fig. 6A depicts a typical H\textsubscript{2}O\textsubscript{2} concentration-dependent absorbance changes curve at 450 nm upon analyzing different concentrations of H\textsubscript{2}O\textsubscript{2}. As higher concentrations of H\textsubscript{2}O\textsubscript{2} were added, the absorbance values increased. Fig. 6B exhibits a good linear relationship between the absorbance intensity and H\textsubscript{2}O\textsubscript{2} concentration from 5 to 140 μmol (R\textsuperscript{2}=0.994). The limit of detection was estimated to be 1.0 μmol from three times the standard deviation corresponding to the blank sample detection, which was lower than that reported in most of the previous reports (Table S2\textsuperscript{*}). To explore the selectivity of the novel H\textsubscript{2}O\textsubscript{2} sensing system, the catalytic reaction was further investigated at the presence of some common impurities such as NaCl, KCl, CaCl\textsubscript{2}, ascorbic
acid, dopamine, cysteine, ClO' and Cr₂O₇²⁻. When the concentrations of inorganic salts were higher than 0.5 M, hemin/WS₂ NSs were observed to aggregate in salt solution because of the charge screening effects.29 Furthermore, when the concentrations of the small biological molecules were lower than 10 mM, they did not disturb the determination of H₂O₂ (Table S3). These results clearly demonstrated that our proposed sensor could be used to detect H₂O₂ in the presence of other oxidants because of its great selectivity. When combined with glucose oxidase (GOx), GOx catalyzed the oxidation of glucose to gluconic acid in HAc-NaAc buffer solution, and oxygen in solution was converted to H₂O₂, which would oxidize TMB to produce a blue-color product via the catalysis of hemin-functionalized WS₂ nanosheets. This indicates that proposed label colorimetric method could also be used for the determination of glucose, which is an important indicator for the diagnosis of diabetes in clinical medicine.17,18 As illustrated in Fig. 7A, the absorption intensity of this solution at 450 nm gradually increased with the increasing of glucose concentration. The linear range for glucose (Fig. 7B) was from 5 to 200 μmol L⁻¹ (R²=0.9983) with a limit of detection of 1.5 μmol L⁻¹, which was more sensitive than that reported in most of the previous reports (Table S2⁹).

In order to further explore the selectivity of this novel glucose sensing system, a comparison of the absorption intensity induced by fructose, lactose and maltose were performed. As illustrated in Fig. S7, the results demonstrated that the absorbance of these glucose analogues was negligible compared with that of glucose even at concentrations as high as 5 mM. This indicates that this sensing system has a high selectivity for glucose detection, which could be attributed to the high affinity of glucose oxidase for glucose. Finally, in order to evaluate the applicability of the proposed method based on the Hemin/WS₂-NSs for the determination of glucose in complex biological samples. The present label-free colorimetric assay was used to quantify glucose in three human serum amyles from healthy individuals. The results of the determination and recovery are shown in Table S4. One observed that the recoveries are ranged from 99.0% to 102%, and the relative standard deviations are from 1.6% to 4.0%. These results indicate the validity of the proposed label-free colorimetric method for the determination of glucose in real biological samples.

4. Conclusions

In summary, we provide the first report that hemin-functionalized WS₂ nanosheets exhibit intrinsic peroxidase-like activity, which follows the typical Michaelis–Menten kinetics and ping-pong mechanism. Meanwhile, as a new support of hemin, WS₂ nanosheets were presented, showing their strong van der Waals force when interacting with plane-like molecules. In addition, the hemin/WS₂-NSs as peroxidase mimetics show several advantages over HRP and other peroxidase nanomimetics, such as ease of preparation, stability, dispersibility, high catalytic efficiency and low-cost. The hemin/WS₂-NSs can be successfully used for label-free, sensitive and highly selective colorimetric detection of H₂O₂ and glucose. In consideration of these advantages, we believe that the new prepared hemin/WS₂-NSs would have potential applications in environmental chemistry, biotechnology and clinical diagnostic.

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