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A thiamine-triggered fluormetric assay for acetylcholinesterase activity and inhibitor screening based on oxidase-like activity of MnO_2 nanosheets

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ABSTRACT

Acetylcholinesterase (AChE) plays an essential role in biological signal transmission, the aberrant expression of which could cause diverse neurodegenerative diseases. Herein, based on the oxidase-like activity of manganese dioxide nanosheets (MnO_2 NSs), we found that MnO_2 NSs could directly oxidize thiamine into intensely fluorescent thiochrome without the need of peroxides. When AChE was introduced, acetylthiocholine could be hydrolyzed to generate thiocholine, which efficiently triggered the reduction of MnO_2 NSs into Mn^{2+}, resulting in the decrease of fluorescence. Owing to the inhibiting effect of tacrine to the AChE activity, the decomposition of MnO_2 was hindered, thus leading to the fluorescence recovery. According to the above mechanism, we constructed a simple, low-cost, label-free, facile and rapid synthetic fluorescent biosensor for highly sensitive and selective detection of AChE activity and screening of its inhibitor. This biosensor obtained a good linear range from 0.02 to 1 mU/mL and an extremely low detection limit of 15 μU/mL for AChE assay, as well as a sensitive screening for tacrine and an excellent applicability in human serum samples. These results suggested that our proposed method would be potentially applied in monitoring the disease progression.

1. Introduction

As a primary and critical enzyme in central nervous system, acetylcholinesterase (AChE) plays crucial roles in biological signal transmission [1]. It can control the levels of neurotransmitter acetylcholine (ACh) at neuromuscular junctions and cholinergic synapses by catalyzing the hydrolysis of ACh into choline and acetate, leading to the termination of the transmission process [2]. The abnormal expression of AChE is significantly related to some neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease, and so on [3]. Much research has attested that the breakdown of ACh in the brain by AChE can accelerate the aggregation of amyloid beta peptides to form fibrils and this process acts a pivotal part in the cause of AD [4–6]. Accordingly, the symptomatic treatment of AD is mainly dependent on the increase of ACh levels for enhancing cholinergic transmission by the effective inhibition of AChE activity [7,8]. Therefore, sensitive and effective detection of AChE activity and screening of its potential inhibitors are of significant importance for biomedical research and early diagnosis and therapy of diseases.

Up to now, a great many methods for AChE activity assay have been established, such as colorimetric [9,10], fluorescent [11–13], electrochemical [14], chemiluminescent [15], and so on. However, most of the above systems have certain limitations and drawbacks. For instance, traditional colorimetric method by using Ellman's reagent suffers from low sensitivity, time-consuming operation and false-positive effect [9]. The electrochemical detection with layer-by-layer assembly can obtain the improved sensitivity, whereas the complex electrode modification results in sophisticated test steps [16]. One of the most attractive approaches is to develop fluorometric determination for AChE activity because of its simplicity, high sensitivity and rapid response. Typically, the fluorescent probes depending on organic dyes show a good sensitivity and selectivity, but their inherent deficiencies including poor stability and insufficient emission intensity limit their development [17]. At the same time, more and more nanomaterials, such as metal nanoparticles [18,19], inorganic quantum dots [20,21], and fluorescent conjugated polymers [22], have also been employed for the detection of AChE activity. Although great achievements have been achieved, the synthetic procedures of conjugated polymers and nanoparticles are

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rather sophisticated, time-consuming and cost-intensive. Some quantum dots have high toxicity, which greatly limited their applications. Therefore, it is still challenging to explore a simple, low-cost, non-toxic, facile and rapid synthetic fluorescent probe for monitoring AChE levels sensitively and selectively.

Thiamine, known as vitamin B1, which is present in many animal and plant tissues, has been extensively employed as non-fluorescent substrate owing to its attractive advantages of high stability, good water solubility, cost effectiveness and easy accessibility [23–25]. It can be easily converted into intensely fluorescent thiochrome depending on the oxidation of thiamine by appropriate oxidants. The most common approach to obtain the fluorescent thiochrome is depended on the oxidation of thiamine by peroxide (e.g., hydrogen peroxide and organic peroxides), mediated by a peroxidase enzyme or an enzyme mimic. For example, Li et al. reported that under the catalysis of horseradish peroxidase (HRP), the thiamine can be oxidized into thiochrome by H_2O_2 with a yield greater than 95% [26]. In addition, other oxidizing agents including hexacyanoferrate(III) [27], cyanogen bromide [28], mercury(II) [29], gold(III) [30] and copper(II) [31] have also been used to oxidize thiamine. To our knowledge, most of these reported oxidants have their own shortcomings such as high toxicity, increased cost, instability, or introducing the formation of other fluorescent species. In recent years, the inorganic nanomaterials with peroxidase-like activity have become a kind of specially appealing materials and have been extensively used in various fields owing to their striking properties of inexpensiveness and good stability as well as tunable catalytic activity [32,33]. However, the application of inorganic nanomaterials used to oxidize thiamine for monitoring enzyme activity has rarely been studied.

Recently, manganese dioxide nanosheets (MnO_2 NSs), as a kind of single-layer inorganic nanomaterial, have aroused significant attention due to their superior physicochemical properties of low toxicity, high specific surface area, good water solubility and biocompatibility [34–36]. Besides, taking advantage of the oxidase-like activity, MnO_2 NSs could be selected as oxidase mimics to directly oxidize substrates for biosensing [37,38].

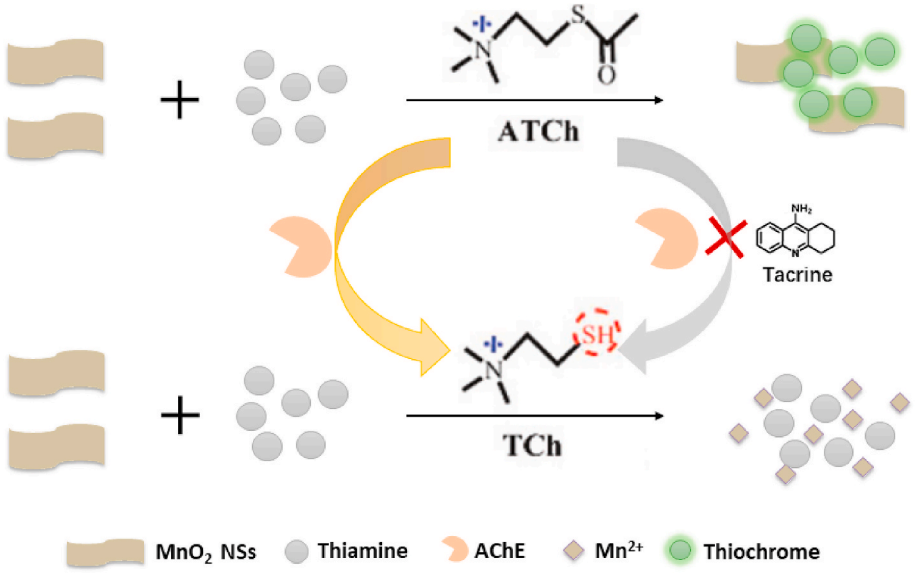
Herein, based on the oxidase-like activity of MnO_2 NSs, we discovered that MnO_2 NSs could directly oxidize thiamine into intensely fluorescent thiochrome. Especially, when acetylthiocholine (ATCh) and AChE were introduced to this system, AChE could catalyze the hydrolysis of ATCh to generate thiocholine (TCh), whereat MnO_2 NSs would be reduced into Mn^{2+} by TCh and lose the oxidase-mimicking activity, accompanied with the subsequent decrease of fluorescent intensity. However, in the presence of tacrine, the activity of the enzyme would be suppressed, which impeded the generation of TCh and destruction of MnO_2 NSs, thus the fluorescence of this system was recovered. According to the above mechanism, we successfully established a simple, low-cost, label-free, facile and rapid synthetic fluorescent biosensor for highly sensitive detection of AChE activity and screening of its inhibitor (Scheme 1). This fluorescent probe exhibits an excellent sensing performance, and the detection limit of AChE is as low as 15 μU/mL, as well as the excellent specificity, sensitive inhibitor screening and real sample test were also obtained. Furthermore, such a low-toxic, good biocompatible and facile-synthesized biosensor would be promising for the potential applications in bioimaging and disease diagnosis.

2. Material and methods

2.1. Reagents and instruments

Thiamine, AChE, acetylthiocholine chloride, tacrine, alkaline phosphatase (ALP), IgG (from human serum), peroxidase, glucose oxidase (GOx), human serum albumin (HSA), lysozyme, catalase and trypsin were purchased from Sigma-Aldrich Corporation (Shanghai, China). Tetramethylammonium hydroxide, glutathione (GSH) and L-cysteine (Cys) were obtained from Aladdin Reagent Company (Shanghai, China). Glucose, threonine and serine were purchased from Dingguo Biotechnology Company (Beijing, China). L-ascorbic acid (AA) was obtained from Macklin Reagent Company (China). H_2O_2 (30%) and manganese chloride tetrahydrate ($MnCl_2{\cdot}4H_2O$) were purchased from Beijing Chemical Works (China). The water used throughout all experiments was purified through a Millipore system. All reagents were analytical grade and used as received.

Fluorescence and absorption spectra were recorded on a Hitachi F-4600 spectrofluorometer (Tokyo, Japan) and a CARY 500 UV−vis−NIR Varian spectrophotometer (CA, USA), respectively. X-ray photoelectron spectroscopy (XPS) was obtained with an ESCALABMK II X-ray photoelectron spectrometer using Al Kα as the exciting source. Transmission electron microscopy (TEM) images were acquired by Tecnai G2 F30 (America). The scanning electron microscopy (SEM) measurements were performed with a XL30 ESEM FEG microscope at an accelerating voltage of 15 kV. Fourier transform infrared (FT-IR) spectra were surveyed using a Bruker Optics VERTEX 70 spectrometer (Ettlingen, Germany) in the transmission mode.



Scheme 1. Schematic illustration of the thiamine-MnO_2 fluorescent probe for acetylcholinesterase activity assay and its inhibitor screening.

2.2. Synthesis of MnO_2 NSs

The single-layer MnO_2 NSs were prepared as reported previously [39]. Briefly, 5 mL of $MnCl_2$ (0.3 M) solution and 10 mL of tetramethylammonium hydroxide (0.6 M) containing 3 wt % of H_2O_2 were mixed as soon as possible. Then, the obtained dark brown suspension was kept at room temperature overnight with vigorous stirring in the open air. MnO_2 NSs were acquired by centrifugation at 12000 rpm for 10 min and washed several times with ultrapure water and methanol. The resulting product was dispersed with 40 mL of ultrapure water and stored at 4 °C for further use.

2.3. Detection of AChE activity by the oxidation of thiamine

For the detection of AChE activity, 50 μL of ATCh (500 μM) and 50 μL of phosphate buffer (PB, 20 mM, pH 7.4) were mixed with 50 μL of AChE at various concentrations (final concentration 0.020, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.80, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 mU/mL, respectively). After incubating for 40 min at 37 °C, 50 μL of MnO_2 NSs (19.8 μg/mL) was introduced. Two minutes later, 50 μL of thiamine (1 mM) was injected into the above mixture solution. After reacting at room temperature for another 20 min, the fluorescence signals of a series of samples were collected from 390 to 600 nm with the excitation wavelength of 370 nm. To investigate the specificity of the proposed biosensor for AChE activity, some interferences were utilized, such as threonine, serine, glucose, human IgG, HSA, ALP, GOx, lysozyme, trypsin, peroxidase, catalase, GSH, AA and Cys.

2.4. Inhibitor screening assay

To investigate the inhibition efficiency of tacrine, 25μL of tacrine at different concentrations (final concentration 0.10, 0.50, 2.0, 5.0, 10, 20 and 50 nM, respectively) and 25μL of AChE (final concentration 0.8 mU/mL) were added into 50 μL of PB (20 mM, pH 7.4) and incubated at 37 °C for 20 min. Then, 50 μL of ATCh solution (500 μM) was injected into the above solution, with an additional incubation time of 40 min at 37 °C. Subsequently, 50 μL of MnO_2 NSs (19.8 μg/mL) was added and incubated for 2 min at room temperature. Finally, the resulting solution was mixed with 50 μL of thiamine (1 mM). After reacting at room temperature for another 20 min, the emission spectra of various samples were measured.

3. Results and discussion

3.1. Characterization of the synthesized MnO_2 NSs

The as-prepared MnO_2 NSs were systematically characterized by using TEM, XPS, FT-IR spectrum and UV–vis spectroscopy. As presented in Fig. 1A, the synthetic MnO_2 NSs exhibited an ultrathin 2D sheetlike structure. In the FT-IR spectrum the MnO_2 NSs presented the characteristic absorbances at around 512 and 430 cm^{-1} (Fig. 1B), which contributed to the Mn–O stretching vibrations [40,41]. The XPS was taken to further identify the formation of MnO_2 NSs. As depicted in Fig. 1C, the as-prepared MnO_2 NSs exhibited two characteristic peaks located at 642.4 eV and 654.1 eV, which corresponded to Mn(IV) $2p_{3/2}$ and Mn(IV) $2p_{1/2}$ of MnO_2, respectively [42]. Besides, the optical property of MnO_2 NSs was verified by UV–vis spectroscopy in Fig. 1D.

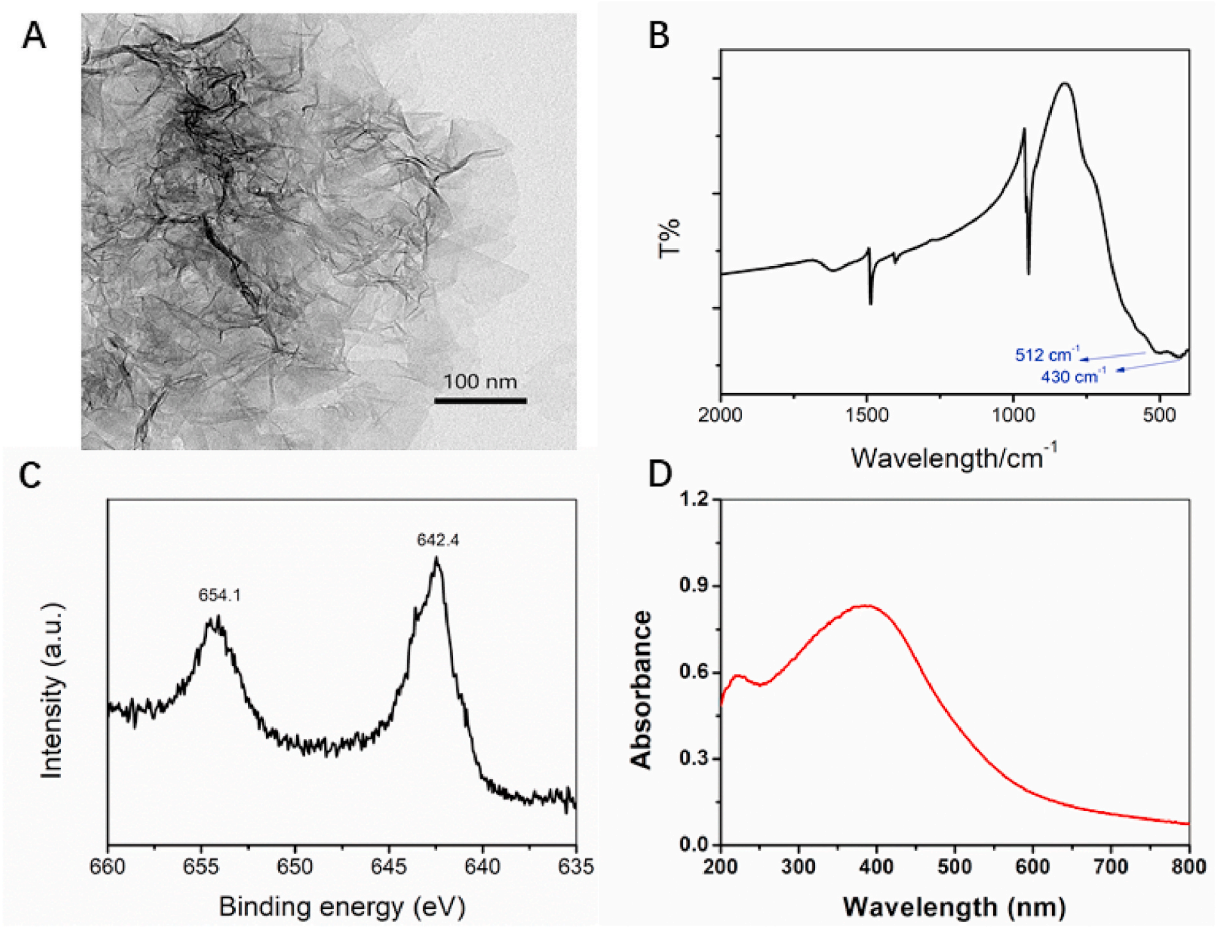


Fig. 1. (A) TEM image, (B) FT-IR spectrum, (C) XPS spectrum and (D) UV–vis absorption spectrum of MnO_2 NSs.

The absorption spectrum showed a broad band in the range of 200–600 nm with a characteristic peak centered at 380 nm, which could be attributed to the d–d transition of Mn^{4+} ions [43]. All those results fully indicated the successful preparation of MnO_2 NSs.

3.2. Oxidation of thiamine by MnO_2 NSs

Thiamine is a nonfluorescent substance, which could be oxidized to intensely fluorescent thiochrome by appropriate oxidants. The previous reports have demonstrated that some enzymes and metal ions could catalyze the oxidation of thiamine into thiochrome in the presence of peroxides [27,44]. Inspired by the oxidase-mimicking activity of MnO_2 NSs, we introduced MnO_2 NSs to directly incubate with thiamine for triggering the fluorimetric reaction. Interestingly, the sole thiamine showed a negligible fluorescent signal, while a very strong fluorescent signal appeared at around 438 nm after the addition of MnO_2 NSs into the thiamine solution (Fig. 2A). Meanwhile, the color of the solution changed from colorless to blue under UV irradiation (the inset in Fig. 2A), indicating that MnO_2 NSs could directly oxidize thiamine into fluorescent thiochrome by virtue of its oxidase-like activity without any other oxidants. To further verify the ability of MnO_2 NSs, we incubated thiamine with different concentrations of MnO_2 NSs, the results in Fig. 2B showed that the fluorescence intensity at 438 nm obviously increased with increasing concentration of MnO_2 NSs. Thus, the fluorescence intensity of the solution could be modulated by the MnO_2 NSs due to its enzyme mimetic activity. Moreover, the SEM and UV–vis absorption spectrum were further utilized to verify the reaction between MnO_2 NSs and thiamine. As can be clearly seen from Fig. 2C and D, the original sheetlike structure of the MnO_2 NSs was broken and the MnO_2 NSs was aggregated and became irregular after incubating with thiamine for 20 min. Meanwhile, the UV–vis absorbances of MnO_2 NSs were slightly decreased, indicating that the MnO_2 NSs might be partially decomposed. Additionally, a new peak appeared at around 370 nm in the UV–vis absorption spectrum which contributed to the characteristic absorbance of thiochrome (Fig. 3A) [45]. When given a long reaction time, such as 24 h, the color of thiamine-MnO_2 mixture solution would change from brown to colorless by naked eyes (Fig. 3B). Besides, the MnO_2 NSs were almost completely decomposed from the SEM images (Fig. S1), as well as, the UV–vis absorbances of MnO_2 NSs were also nearly disappeared and only the absorption spectrum of fluorescent thiochrome was retained (Fig. 3A). Simultaneously, the bright blue fluorescence could be clearly seen under UV irradiation (Fig. 3B). All of the above phenomena convincingly proved that the thiamine could be oxidized into intensely fluorescent thiochrome by MnO_2 NSs. Furthermore, the optical properties of fluorescent thiochrome were also characterized. When excited at different wavelengths in the range of 330–390 nm, the fluorescent thiochrome exhibited only one emission peak at around 438 nm (Fig. 3C), which suggested that the fluorescence emission of thiochrome was excitation-independent and the maximum excitation was located at 370 nm. In addition, the excitation and emission spectra of thiochrome were shown in Fig. S2.

Moreover, considering the effects of the pH and reaction times on the fluorescence intensity of thiamine-MnO_2 system, we investigated the fluorescence changes of this system when the pH values ranged from 4.0 to 12 and the fluorogenic reaction times varied from 10 min to 240 min. As depicted in Fig. 3D, the fluorescence intensity of thiamine-MnO_2

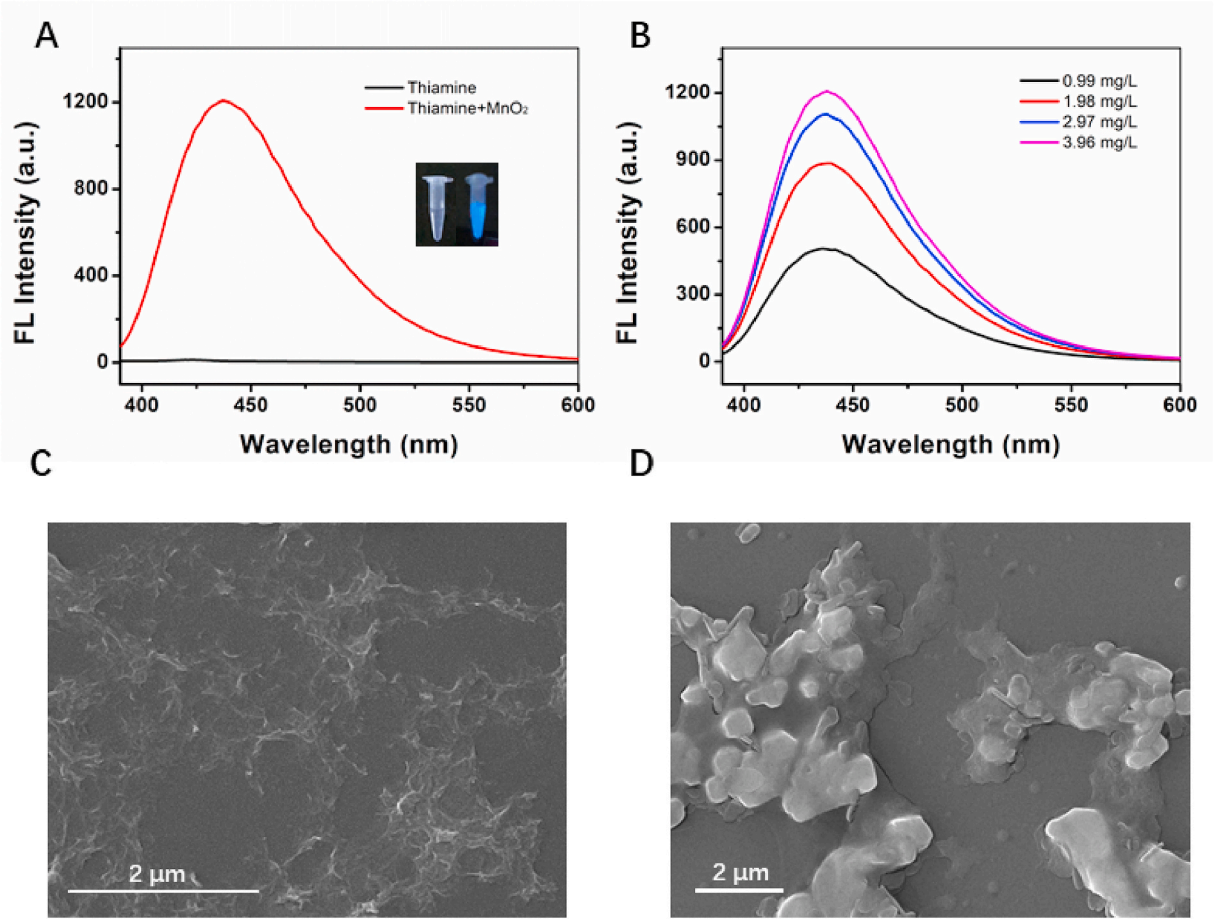


Fig. 2. (A) Fluorescence emission spectra of sole thiamine and thiamine-MnO_2 system, the inset showed the corresponding photographs under UV lamp at 365 nm. (B) The fluorescence emission spectra of thiamine with different concentrations of MnO_2 NSs. SEM images of (C) MnO_2 NSs and (D) MnO_2 NSs after incubating with thiamine for 20 min.

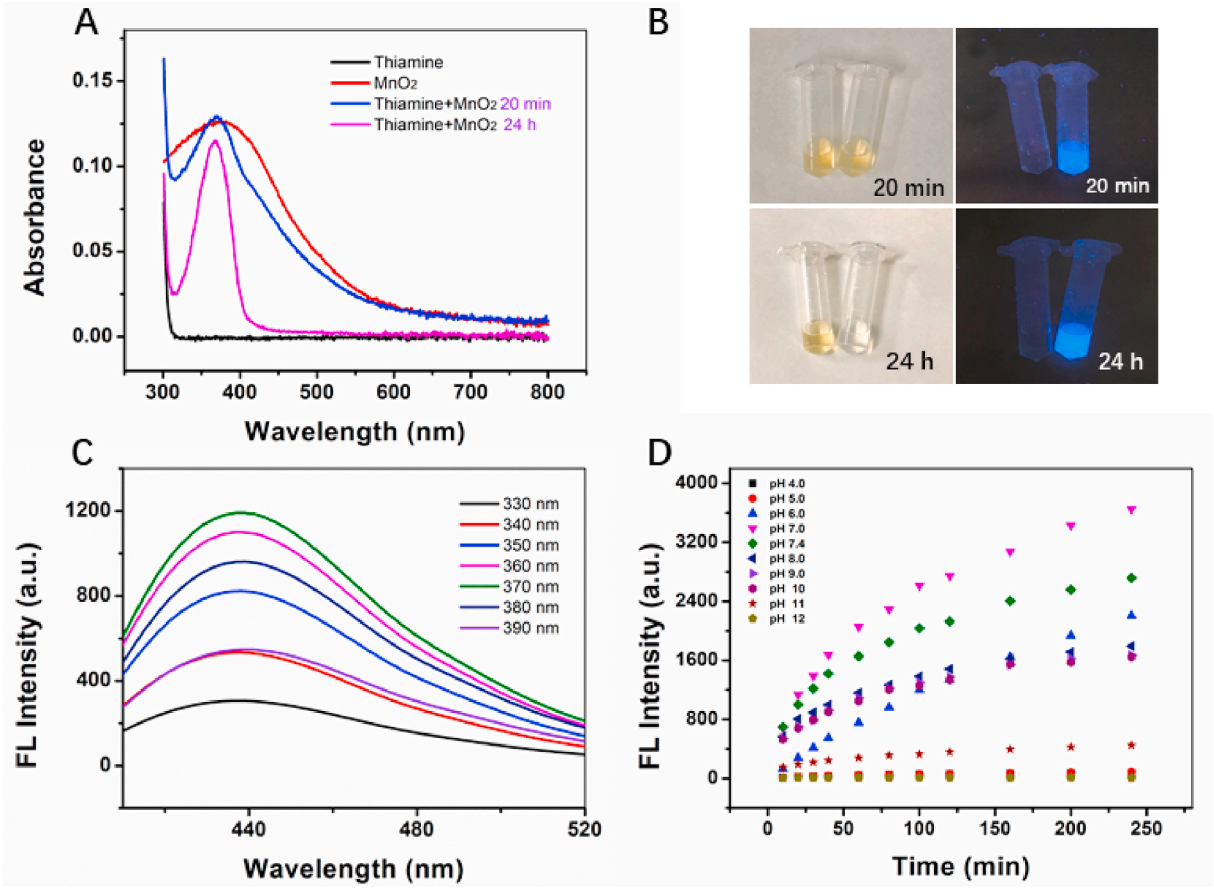


Fig. 3. (A) UV–vis absorption spectrum of different substances. (B) The photos under room light of sole MnO_2 NSs (left) and thiamine-MnO_2 system (right) for incubating 20 min (top left) and 24 h (bottom left), as well as the photos under UV lamp at 365 nm of sole MnO_2 NSs (left) and thiamine-MnO_2 system (right) for incubating 20 min (top right) and 24 h (bottom right). (C) Fluorescence signals of thiochrome at various excitation wavelengths. (D) Fluorescence intensity as a function of incubation time under different pH values.

system was the highest at around pH 7.0, and the system exhibited the lower fluorescence intensity with the higher pH values (pH > 11.0) in alkaline condition or the lower pH values (pH < 5.0) in acidic condition. Besides, the fluorescence intensities at 438 nm of thiamine-MnO_2 system gradually increased along with the increasing reaction times in various pH values, and a very short reaction time can oxidize thiamine to reach a specially high fluorescence intensity around the neutral condition, indicating that the neutral condition was benefit for the reaction of MnO_2 NSs and thiamine. Thus, taking the physiological environment and time-effectiveness into account, we chose pH 7.4 as the detection pH value and 20 min as the fluorogenic reaction time of this system in the following experiments.

3.3. *The principle for fluorimetric determination of AChE*

AChE could catalyze the hydrolysis of ATCh to produce TCh, which triggered the reduction of MnO_2 into Mn^{2+} and resulted in the decomposition of MnO_2 NSs. As shown in eqn (1), TCh was oxidized into

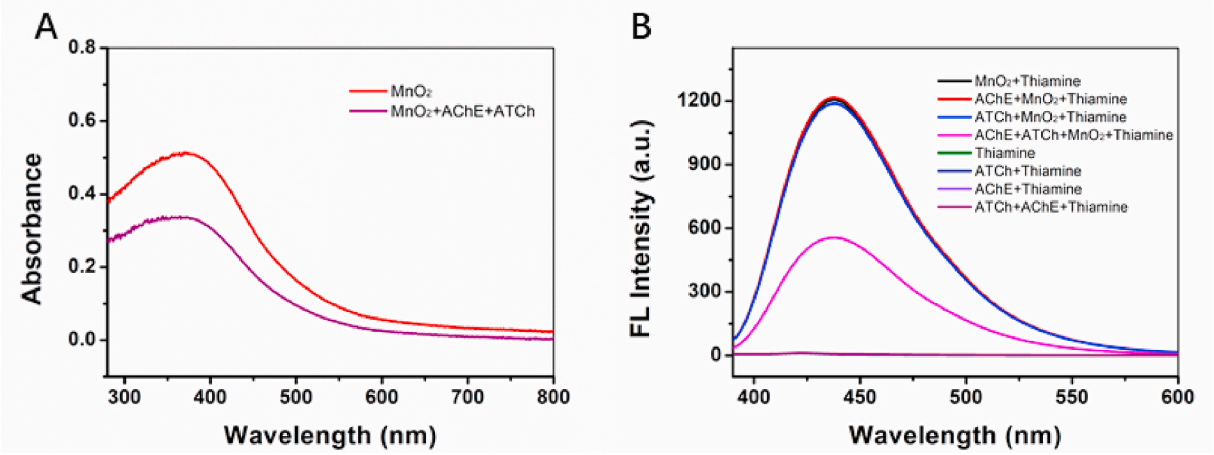
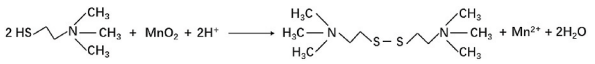


Fig. 4. (A) UV–vis absorption spectra of MnO_2 NSs in the absence and presence of TCh. (B) Fluorescence emission spectra of different solutions.

thiocholine disulfide in this redox reaction simultaneously [46].



(1)

The interaction between MnO_2 and TCh was demonstrated by measuring the UV–vis absorption spectra of MnO_2 NSs in the absence and presence of ATCh and AChE. As can be seen in Fig. 4A, the characteristic absorption peak at 380nm of MnO_2 NSs was obviously decreased when the hydrolysate (TCh) was added. The result suggested that the MnO_2 NSs could be easily reduced by TCh.

To evaluate the feasibility of the biosensor for the detection of AChE, the fluorescence spectra of different solutions were measured. As shown in Fig. 4B, the sole thiamine and the mixture of thiamine and ATCh exhibited negligible fluorescent signal at 438 nm. Similarly, when AChE was added into the sole thiamine solution and the mixed solution of thiamine and ATCh, respectively, there were almost no fluorescence signals under the same conditions, indicating that ATCh, AChE and the mixture of ATCh and AChE would not catalyze the oxidation of thiamine into fluorescent substance. As the MnO_2 NSs were introduced to the sole thiamine solution, the fluorescence intensity at 438 nm was significantly improved because of the strong oxidase-like activity of MnO_2 NSs. Besides, the fluorescence signals remained almost unchanged after ATCh or AChE was added into the mixed solutions of thiamine and MnO_2, which demonstrated that the ATCh or AChE had almost no effect on the oxidation reaction between MnO_2 and thiamine. After the addition of MnO_2 and thiamine into the mixture solution of AChE and ATCh, the obtained fluorescence signal was obviously decreased owing to the reduction of MnO_2 NSs into Mn^{2+} by TCh, which is the hydrolysis product of AChE by using ATCh as substrate. Thus, the above results essentially demonstrated that our proposed fluorescent biosensor could be used for the detection of AChE activity.

3.4. Optimization of experimental conditions

To obtain the sensitive sensing of AChE activity based on the thiamine-MnO_2 probe, the experimental condition optimizations were systematically investigated, including the enzymatic reaction time and the concentrations of thiamine, MnO_2 NSs and ATCh.

Obviously, the concentrations of thiamine and MnO_2 NSs have a great effect on the fluorescent signals of thiamine-MnO_2 system. As shown in Fig. S3, the fluorescence intensity at 438 nm increased rapidly with increasing concentrations of thiamine and then the rate of fluorescence change slowed down. Considering the fluorescence intensity and the rate of fluorescence change as well as the cost-effectiveness, we chose 200 μM as the optimal concentration of thiamine for the subsequent experiments. With the increase of MnO_2 concentration, the fluorescence intensity of thiamine-MnO_2 system progressively increased. When the MnO_2 concentration increased to 3.96 mg/L, the enhanced fluorescence intensity was slowed down. And then the highest fluorescent signal was obtained when the concentration of MnO_2 NSs was up to 4.95 mg/L. After that, the fluorescence intensity of this system started to decrease (Fig. S4). The phenomenon indicated that the excessive MnO_2 NSs may have a quenching effect on the fluorescence of the thiochrome, which was the oxidation product of thiamine by MnO_2. Therefore, we selected 3.96 mg/L as the most appropriate MnO_2 NSs concentration.

The enzymatic reaction time between ATCh and AChE was also optimized. As depicted in Fig. S5, the fluorescence intensity of the system decreased gradually with the increase of incubation time. After 40 min, the fluorescence intensity reached minimum and remained constant after that, indicating that the reaction achieved equilibrium. Hence, 40 min was taken as the optimal reaction time.

In addition, the concentration of enzymatic substrate (ATCh) was further optimized. The relationship between the ATCh concentration and the fluorescence intensity ratio $(F_0 - F)/F_0$ (F_0 and F represent the fluorescence intensity of ATCh-MnO_2-thiamine system in the absence and presence of AChE, respectively.) was shown in Fig. S6. The $(F_0 - F)/F_0$ value sharply increased along with increased ATCh concentration, and then reached a balance when the ATCh concentration was up to 100 μM. Thus, 100 μM was adopted as the optimal concentration of enzymatic substrate.

3.5. Quantitative detection of AChE

The AChE activity was investigated under the above optimal conditions. The fluorescence intensities at 438 nm of the sensing system decreased gradually along with AChE concentrations ranging from 0 to 5 mU/mL. (Fig. 5A). As displayed in Fig. 5B, the fluorescence intensity ratio $(F_0 - F)/F_0$ was gradually enhanced along with the increase in AChE concentration from 0.02 mU/mL to 5 mU/mL. It could be observed that the fluorescence intensity ratio was closely related to the AChE activity and the $(F_0 - F)/F_0$ correlates linearly well with the AChE concentration in the range of 0.02–1 mU/mL. The regression equation was expressed as $(F_0 - F)/F_0 = 0.0869 + 0.5087\ C_{[AChE]}$ (mU/mL), $R^2 = 0.98$. The detection limit for AChE was calculated to be 15 μU/mL. This value was comparable to or even better than those of previous assays (Table S1), suggesting that our proposed biosensor was suitable for AChE activity detection.

3.6. Selectivity of thiamine-MnO_2 probe for AChE detection

To further evaluate the selectivity of the proposed fluorescent probe toward AChE, the specificity test was carried out by measuring the fluorescence intensities of the blank and various other interferences including threonine, serine, glucose, human IgG, HSA, ALP, GOx, lysozyme, trypsin, peroxidase, catalase, GSH, AA and Cys. Obviously, as presented in Fig. 5C, the fluorescence signals at 438 nm of the sensing system were significantly decreased when only AChE existed, while the interfering substances had a negligible effect on the fluorescence intensity. The above results revealed that our proposed biosensor presented excellent specificity toward AChE sensing.

3.7. Determination of AChE in human serum

To investigate the practicality of the proposed sensor in real samples, AChE activity was measured in the 20000-fold diluted human serum samples to avoid background and biothiol interference. As shown in Table S2, taking into consideration the dilution factors of the serum samples, the cholinesterase (ChE) was determined to be 7660–8320 mU/mL, which was similar to that reported by Yan et al. for human serum [46]. Additionally, the standard additional method showed the recovery of 90.33–99.74 %. These results illustrated that our proposed thiamine-MnO_2 probe possessed great potential to monitor AChE activity in biological samples.

3.8. Inhibitor screening for AChE

In order to verify the potential application of the biosensor in the field of screening AChE inhibitor, tacrine, a common AChE inhibitor and an important medicine for the treatment of Alzheimer's disease, was selected as a model analyte to evaluate the feasibility. As displayed in Fig. 6A, a strong fluorescence signal was obtained when the MnO_2 NSs were added to the thiamine-ATCh mixture solution, and the following addition of AChE into the above ATCh-MnO_2-thiamine system caused an obvious decrease of fluorescence intensity. However, when AChE mixed with tacrine (50 nM) was added to the ATCh-MnO_2-thiamine system, the resultant mixture exhibited the similar fluorescence signal to that of ATCh-MnO_2-thiamine system, which strongly demonstrated the AChE hydrolysis reaction was effectively inhibited by tacrine. As presented in Fig. S7, the fluorescence intensities at 438 nm gradually increased with the concentrations of tacrine ranging from 0.10 to 50 nM. Generally, the increased fluorescence intensities of the proposed system further confirmed the correlation between the inhibition efficiencies and tacrine

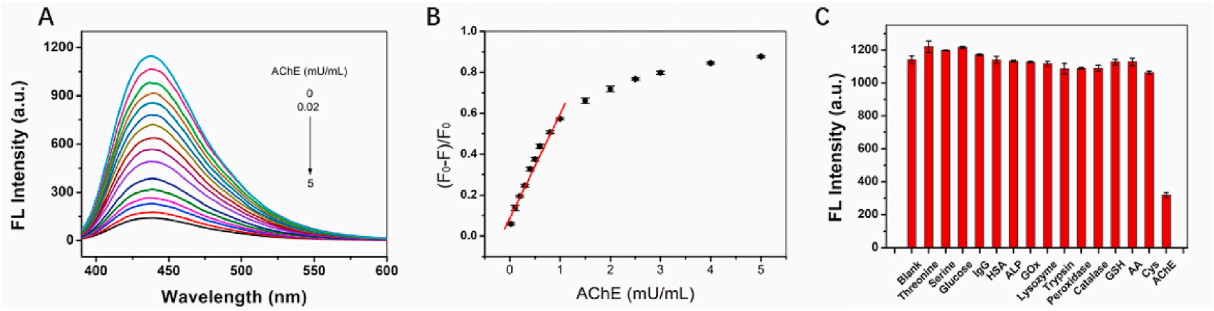


Fig. 5. (A) Fluorescence emission spectra at various concentrations of AChE. The final concentrations of AChE are 0, 0.020, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.80, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0 and 5.0 mU/mL, respectively. (B) The fluorescence intensity ratio (F_0 - F)/F_0 at 438 nm versus different AChE concentrations. (C) Fluorescence intensities of the sensing system in the presence of various amino acids or sugars (1 mM), proteins (0.5 μg/mL), enzymes (20 mU/mL) and other small molecules. The concentrations of GSH, AA and Cys were all 1 μM. The concentration of AChE was 2 mU/mL.

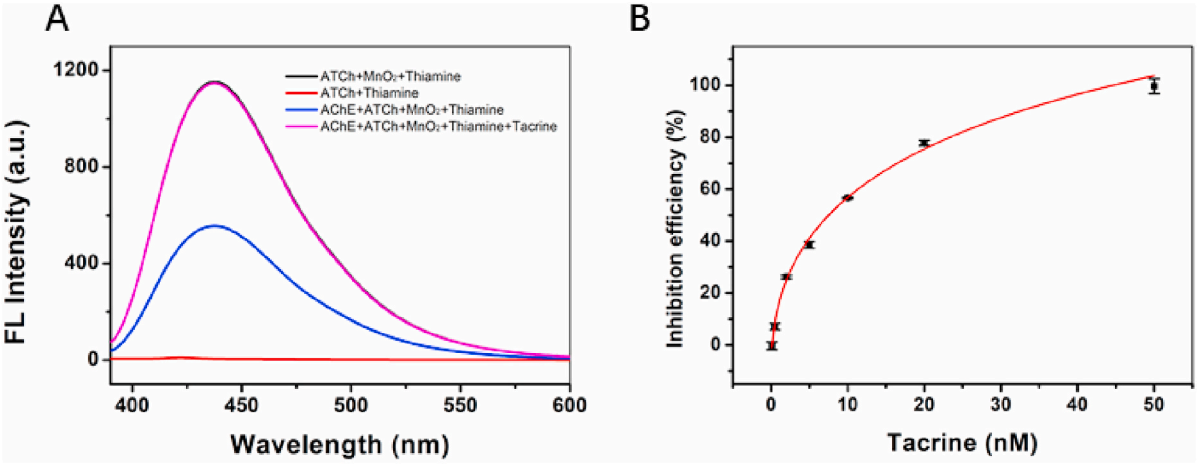


Fig. 6. (A) Fluorescence emission spectra of different solutions. (B) Inhibition efficiencies versus the concentrations of tacrine (0.10, 0.50, 2.0, 5.0, 10, 20 and 50 nM, respectively).

concentrations. The IC_{50} value (concentration of inhibitor when AChE activity is suppressed by 50%) was estimated to be 7.5 nM from the curve in Fig. 6B. The above results indicated that this established biosensor could be employed to screen the potential AChE inhibitor.

4. Conclusion

In summary, based on the oxidase-like activity of MnO_2 NSs, we discovered that MnO_2 NSs could directly oxidize thiamine into intensely fluorescent thiochrome. By virtue of this fluorogenic reaction, we further constructed a MnO_2-thiamine fluorescent probe for highly sensitive detection of AChE activity and screening of its inhibitor by taking advantage of the reduction of MnO_2 NSs into Mn^{2+} by TCh, which is the AChE-catalysed product with ATCh as the substrate. Compared with traditional thiamine-H_2O_2-based sensing systems, MnO_2 NSs can efficiently oxidize thiamine without additional oxidizing agents (e.g., hydrogen peroxide and organic peroxides). In addition, on account of the strong oxidizing ability of MnO_2 NSs, even low concentration of MnO_2 NSs can oxidize thiamine to reach a specially high fluorescence intensity within a short time (20 min), resulting in a rapid and cost-effective sensing system. Moreover, the proposed biosensor achieved an extremely low detection limit of 15 μU/mL for AChE assay, as well as the excellent specificity, sensitive inhibitor screening and real sample test were also obtained. We envision that such a novel biosensor has great potential in biosensing and biological applications.

Author statement

Ting Xiao: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing. Shuang Wang: Formal analysis, Writing - review & editing. Mengxia Yan: Writing - review & editing. Jianshe Huang: Supervision, Resources, Writing - review & editing, Project administration. Xiurong Yang: Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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