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Research paper

Thiol-triggered disaggregation-induced emission controlled by competitive coordination for acetylcholinesterase monitoring and inhibitor screening

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ABSTRACT

Acetylcholinesterase (AChE) is an important biomarker of many neurotransmitter-associated diseases and can be used to screen potential drugs and diagnose poisoning by chemical warfare agents and pesticides, and thus the accurate detection of AChE is of great practical significance. In this work, thiol-triggered disaggregation-induced emission through competitive interaction among 3,4,9,10-perylenetetracarboxylic acid (PTCA), copper ion and glutathione is identified, and a reliable fluorometric assay for acetylcholinesterase (AChE) activity is developed on the basis of this molecular switch. It is found that PTCA in neutral and alkaline solution exhibits very bright green fluorescence, and its quantum yield is up to 78% in alkaline solution. The presence of Cu^{2+} leads to the formation of PTCA/Cu(II) nanoaggregates and the quenching of the fluorescence, but the fluorescence can be fully switched on after the introduction of equivalent amount of thiol-containing compounds such as glutathione. A reversible fluorescence switch can be run in this way by the alternative introduction of Cu^{2+} and GSH. This switch enables the detection of AChE in the signal turn-on mode when acetylthiocholine is used as the substrate. Quantitative measurement of AChE level with detection limit as low as 4 U/L and a broad linear scope ranging from 15 to 500.0 U/L is achieved based on the developed assay. The inhibitor screening function was also assessed using tacrine as the example, and the assay performs well in the screening tacrine. This work proposed thiol-triggered disaggregation-induced emission mediated by competitive interaction, and provides a sensitive detection method for AChE level and its inhibitor screening.

1. Introduction

Acetylcholinestrase (AChE), as one key member of cholinesterase enzyme family, plays a vital role in a range of important areas including neutobiology, toxicology and pharmacology. Its main function in biology is found to efficiently catalyze the hydrolysis of the neutrotransmitter acetylcholine into choline, and thus it serves the role of keeping acetylcholine level of central nervous system in the normal range [1]. Abnormally elevated AChE can accelerate the assembly of amyloid β peptides into amyloid fibrils, and consequently leads to Alzheinmer's disease [2]. As a result, effective AChE inhibitors are useful to treat Alzheimer's disease and can be used as the primary drugs [3]. It is evident that a wide variety of chemical warfare agents and pesticides are able to efficiently inhibit AChE activity, and this kind of poisoning can be accurately diagnosed by measuring the AChE activity [4,5]. Therefore, the development of highly sensitive, reliable assays for AChE and its inhibitor screening method has been always becoming an urgent and significant task in clinical diagnosis and drug development.

A detailed history and new assays for cholinesterase activity and inhibition has been reviewed by Miao et al. [6], and this review presented a variety of methods, such as spectrometric assays, thin-layer chromatographic assays, radiometric assays and calorimetric assays, for the monitoring of AChE activity. As stated in the review, fluorometric assays are more desirable to quantify AChE activity due to their higher sensitivity among these spectrometric assays although the two classical methods are based on Ellman's reagent and quantification of hydrogen peroxide produced by oxidation of the AChE-induced choline. For this reason, a number of fluorometric assays for AChE activity were developed based on molecular fluorophores and luminescent nanomaterials. Although luminescent nanomaterials, such as carbon quantum dots [7–10], noble

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metal nanoclusters [11–13] and silica nanoparticles [14], have their inherent advantages including good aqueous solubility, excellent biocompatibility, and stable photobleaching resistance in detection of AChE activity, they also suffer from their specific drawbacks. The relatively low luminescence quantum yield and significant variations of nanomaterials from different patches frequently leads to the problems from sensitivity and reproducibility of the developed assays in practical samples. This issue drove us to move back to fluorometric assays based on molecular fluorogens. Among several detection strategies for AChE assays, enzymatic reaction induced aggregation via electrostatic interaction is the most frequently used based on different molecular fluorogens, but it may be significantly affected by the ionic strength of practical samples [15–18]. In comparison with the aforementioned sensing strategy, reaction-based probes have much high stability to slight environmental alteration. These probes undertake a nucleophilic substitution or addition to light up or quench the fluorescence of resulting compounds when thiocholine is generated under the catalysis of AChE [19–21]. However, the acquisition of these fluorescent probes requires experienced synthesis skill and complicated purification. These high requirements largely limit the application of these probes in practical samples.

Perylene derivatives, as traditional luminescent molecular materials, have intense molar absorptivities and high fluorescence quantum yields, and thus frequently used as fluorescent probes [22]. Grisci et al. [23] developed fluorescent supramolecular ensembles assembled by a perylene derivative and surfactant for AChE activity monitoring. Liao et al. [24] combined aggregation-induced quenching effect and the competitive interaction among choline-modified perylene, silver ion and anionic polymer PDI to quantify AChE activity. However, the former assay lacks sufficient sensitivity while the latter assay may be subjected to the slight variation of ionic strength. To overcome these drawbacks of the preceding assays, herein we reported a fluorescence switch of perylenetetracarboxylic acid controlled by competitive coordination reaction, and a sensitive fluorometric assay for AChE based on this enzymatic reaction triggered molecular switch. 3,4,9,10-Perylenetetracarboxylic acid (PTCA) was first adopted as the fluorogen because it possesses very bright green fluorescence in weakly alkaline solution, and its carboxylic acid groups can serve as the recognition unit for copper ions. The presence of copper ion leads to self-assembly of PTCA accompanying the fluorescence switch-off, but the introduction of thiol-containing compounds results in the disaggregation of the ensembles with the fluorescence signal turn-on. This fluorescence switch is further utilized to develop a fluorometric assay for AChE activity using acetylthiocholine as the substrate. Its inhibitor screening function was also tested using tacrine as an example.

2. Experimental

2.1. Materials and reagents

Triple-distilled water was used throughout the experimental process. 3,4,9,10-Perylenetetracarboxylic acid dianhydride, copper(II) nitrate ($Cu(NO_3)_2$), acetylthiocholine iodide (ATCh), glutathione (GSH), homocysteine (Hcy), cysteine (Cys), tacrine and 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES) were purchased from Aladdin Ltd. (Shanghai, China). Acetylcholinesterase (AChE, EC 3.1.1.7, 2 kU) and new born calf serum were bought from Sigma-Aldrich (Shanghai, China). The concentration of 2-[4-(2-hydroxyethyl)-1-piperazinyl] ethanesulfonic acid buffer solution (HEPES, pH 7.4) was 10.0 mM. All reagents were of analytical grade and without any further purification.

2.2. Synthesis of 3,4,9,10- perylenetetracarboxylic acid (PTCA)

A certain amount of 3,4,9,10-perylenetetracarboxylic dianhydride (0.0392 g, 0.1 mmol) was added into 100 mL of NaOH solution (0.6 mmol). The resulting mixture was refluxed for 2 h with vigorous stirring. After all the substance was dissolved, the mixture solution was cooled down to room temperature. An excess of concentrated hydrochloric acid (10 mL) was added into the preceding solution, and then red precipitate formed. The final product was obtained after washing and drying.

2.3. Thiol-triggered diaggregation-induced emission via the competitive coordination among PTCA, Cu^{2+} and GSH

A certain amount of PTCA (0.025 μM) in HEPES buffer solution was mixed with different amounts of Cu^{2+} in the range of 0.0–37.5 μM, and then the mixtures were monitored by fluorescence spectrometer at the excitation wavelength of 468 nm. The resulting mixture was continuously mixed with different concentrations of GSH in the range of 0.0–51.0 μM, and then the fluorescence was recorded at the optimal excitation.

2.4. Fluorometric assay for AChE activity and inhibitor screening

For optimization of added copper ion amount for fluorescence quenching by copper (II) ions, the fluorescence intensity of the mixtures containing PTCA (0.025 μM) and varying amounts of Cu^{2+} was monitored using fluorescence spectrometer at the optimal excitation wavelength. For the AChE assay, the fluorescence of PTCA was first quenched by adding 4.0 μL of copper (II) solution (10.0 mM) into 2.0 mL of PTCA solution (0.025 μM, HEPES, pH 7.4). Then, a certain amount of the mixture of ATCh (0.25 mM) and AChE (50.0–500.0 U/L) was introduced to the above mixture, and then the mixture was incubated for different time. The fluorescence was recorded after different incubation time of 0–30 min. Matrix effect was assessed using 200-fold dilution of calf serum in the similar way to the above procedure.

For tacrine, different concentrations of tacrine from 0.0 μM to 20.0 μM was mixed with AChE (80.0 U/L) in advance. The as-mixed solutions containing tacrine and AChE were separately added into 2.00 mL of assay solution containing PTCA (0.025 μM), Cu^{2+} (25.0 μM) and ATCh (250.0 μM). Then the fluorescence spectra of the mixture were recorded respectively.

2.5. Characterization methods

The morphologies of PTCA/Cu(II) nanoaggregates were characterized by transmission electron microscopy (TEM), which was performed on a JEOL-2100F instrument with an accelerating voltage of 200 kV. ^1H NMR spectrum for PTCA was conducted on a Bruker AV400 Nuclear Magnetic Resonance spectrometer. The UV–vis spectra were recorded on a PerkinElmer Lambda 950 spectrometer. The photofluorescence spectra were conducted on a PerkinElmer LS-55 fluorescence spectrometer. Time-resolved fluorescence decay tests were performed using Edinburgh Instrument Model FLS980 fluorescence spectrometer.

3. Results and discussion

3.1. Principle of thiol-triggered disaggregation-induced emission and its application in AChE activity monitoring and inhibitor screening

As shown in Scheme 1, 3,4,9,10-perylenetetracarboxylic acid was selected because it can exhibit very intense green fluorescence