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Hybridization chain reaction and DNAzyme-based dual signal amplification strategy for sensitive colorimetric sensing of acetylcholinesterase activity and inhibitor screening in rat blood

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ABSTRACT

We have constructed a novel colorimetric sensing platform for quantitative detection of acetylcholinesterase (AChE) activity and its inhibitor (donepezil) in rat blood, which integrates the signal amplification of DNAzyme and hybridization chain reaction (HCR) with the assembly of gold nanoparticles (AuNPs). Herein, in the presence of AChE and its substrate acetylthiocholine (ATCh), the sensing system exhibits a dramatic decrease in absorbance at 522 nm, where AChE hydrolyzes ATCh into thiocholine, the resulting thiols capture Cu^{2+} from DNAzyme, and then the substrate strand of DNAzyme acts as an initiator to trigger HCR process. The HCR products can induce the assembly of AuNPs via DNA hybridization, accompanying by a sharp color-change from red to blue. When donepezil is added, the enzymatic activity of AChE is suppressed, resulting in an increase in the absorbance at 522 nm. Under optimal conditions, the colorimetric sensing platform shows sensitive responses to AChE and donepezil in the range of 0.02–1.5 mU mL^{-1} and 0.5–100 nM, respectively. The detection limits of AChE and donepezil are as low as 5 μU mL^{-1} and 0.5 nM, respectively. Owing to high sensitivity of the proposed method, various nonspecific interactions can be eliminated with a sufficient dilution, indicating that our strategy has great potential for practical application in neurobiology and pharmacology fields.

1. Introduction

The assay of enzyme activity is of great importance because it plays important role in human (or animal) health. Acetylcholinesterase (AChE), as a primary cholinesterase in the central nervous system, is involved in nerve transmission processes [1,2]. The chief biological function of AChE is to regulate the levels of neurotransmitter acetylcholine by hydrolyzing it into choline, which is necessary to allow a cholinergic neuron to return to its resting state after activation [3,4]. Moreover, many neurodegenerative diseases, including Parkinson's disease, Alzheimer's disease and Huntington's disease, are closely related with the unbalanced transmission of cholinergic by abnormal AChE [5,6]. Monitoring AChE activity gains great attention due to its crucial role in the development of new cholinesterase inhibitors for the palliative treatment of Alzheimer's disease and myasthenia gravis. Up to now, various strategies have been developed to monitor AChE activity, including the traditional and improved Ellman methods [7,8], fluorescent assays [9], and chemiluminescent [10], electrochemical methods [11]. However, it is still highly desirable to explore novel strategies for monitoring AChE activity.

DNAzymes, a kind of catalytic DNA molecules, depend on specific metal ions (e.g., Hg^{2+}, Pb^{2+}, Cu^{2+} and Zn^{2+}) as cofactors and account for specific cleavage sites of the substrate strand of DNAzyme [12,13]. DNAzymes have been reported for a variety of applications, including assays to detect metal ions in environmental contaminants, DNA computing, nanowire production and drugs in preclinical models of cancer [14,15]. Inspired by the gold nanoclusters (AuNCs)-11-MUA-Cu^{2+} ensemble-based fluorescence turn-on assay for AChE activity, AChE-catalyzed hydrolysis product could seize and capture metal ions through the well-known preferential binding affinity of thiol groups to metal ions [16,17]. Thus, metal-selective DNAzymes can be applied to develop a sensitive platform for AChE activity assay. Additionally, to further enhance the sensitivity of the sensing system, the development of multiple signal amplification strategies is highly desirable. Various nucleic acid amplification techniques, including polymerase chain reaction (PCR), strand displacement amplification (SDA), hybridization

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chain reaction (HCR), nicking enzyme-assisted signal amplification and rolling-circle amplification (RCA), have been employed to improve the detection sensitivity of low abundance biomarkers [18–20]. Among them, HCR has evolved as a fascinating strategy due to its enzyme-free feature, autonomous protocol and efficient isothermal amplification capability [21,22]. HCR is an enzyme-free process in which the polymerization of oligonucleotides can form long nicked dsDNA molecule with a cascade of hybridization events triggered by an initiator. The HCR products can be switched into fluorescence, electrochemical signal, chemiluminiscence or colorimetric readouts [23–26].

Gold nanoparticles, as an ideal colorimetric signal reporters, have been widely employed to construct a sensing platform due to their remarkably high extinction coefficients and strongly distance-dependent photonic properties [27]. Previously, some sensitive colorimetric assays based on AuNPs and HCR were developed [28,29]. For example, Wang's group developed a AuNP-based colorimetric strategy through catalyzed hairpin assembly [30], in which the AuNP aggregation is induced by cross-linking of DNA-functionalized gold nanoparticles (AuNPs-DNA). Our group reported a colorimetric sensing strategy for biomolecule assay, which integrated the signal amplification of the HCR with the assembly of AuNPs through triplex formation [31]. Inspired by the aforementioned work, we herein propose a novel colorimetric strategy for sensitive detection of AChE and its inhibitor in rat blood, which is based upon the dual amplification of Cu^{2+}-specific DNAzyme and HCR. With the aid of AuNPs-DNA probe, AChE activity can be transferred to the color change of AuNPs solution. When its inhibitor (donepezil) is added, the enzymatic activity of AChE is effectively inhibited, so the well-dispersed AuNPs solution is observed again. Therefore, the AChE activity and the concentration of donepezil can be estimated by observing the color change with naked eyes or scanning the absorption spectra of the AuNPs.

2. Experimental

2.1. Materials and chemicals

The analytical chemicals including AChE, ATCh, donepezil, 5,5′-dithiobis(2-nitro-benzoic acid) (DTNB), bovine serum albumin (BSA), cholesterol oxidase (CHO), glucose oxidase (GOx), horseradish peroxidase (HRP), lysozyme, urokinase, cysteine, arginine, glucose and fructose were purchased from Sigma-Aldrich. Chloroauric acid ($HAuCl_4{\cdot}4H_2O$), trisodium citrate and other inorganic salts were obtained from Aladdin Industrial Corporation. Ultrapure water (18.2 MΩ cm) obtained from a Milli-Q water purification system (Millipore Co., USA) was used in all experiments. All DNA oligonucleotides used in this study were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and the sequences were listed in Table S1. Sprague-Dawely (SD) rats were obtained from the Animal Experimental Center of Sun Yat-Sen University (Guangzhou, China).

2.2. Apparatus

The absorption spectra were recorded with a UV-1750 UV–vis spectrophotometer (Shimadzu, Japan) at room temperature. Characterizations of transmission electron microscopy (TEM) were performed on a JEM-3010 transmission electron microscope. The diameter distribution of gold nanoparticles in solutions was estimated from Dynamic light scattering (Nano ZS/Mastersizer 2000E, Malvern Instruments) at room temperature. The images of gel electrophoresis were analyzed by the Gel Image Analysis System (JY02S, Beijing, China). All pH values were measured with a model pHs-3e meter.

2.3. Detection of AChE activity

For AChE activity assay, freshly prepared AChE (dissolved in pH 8.0 Tris–HCl buffer, 50 μL) with different activities was mixed with 20 μL of ATCh (5.0 μM) and 5 μL of Cu^{2+} (100 nM). The solutions were incubated at 37 °C for 30 min. Meanwhile, Cu^{2+}-specific DNAzyme was formed on the basis of the reaction between substrate strand and enzyme strand. A mixture containing substrate strand and enzyme strand at an equimolar level was initially dispersed into 50 mM HEPES buffer (pH 7.4, 1.50 M NaCl), and then incubated for 100 min at 37 °C. The residual Cu^{2+} in the reaction mixture of AChE and ATCh reacted with the above-prepared Cu^{2+}-specific DNAzyme for 60 min at 37 °C to cleave the substrate strand. Then, a mixture containing 100 nM H1 and 100 nM H2 was added and incubated for 120 min at 37 °C to form a long-nicked duplex DNA. A volume of 100 μL of as-prepared AuNPs-DNA probe (4 nM) was added. After thoroughly mixing and incubating for about 8 h at room temperature (25 °C), the color changes of the AuNPs solution and the corresponding UV–vis absorption spectra were recorded. For the rat blood AChE assay, the serum samples were first diluted 10,000 times, then the prediluted serum samples were detected under the conditions identical to those described above. It should be noted here that the serum samples were assayed only by a simple dilution without any tedious pretreatments.

2.4. Procedures for donepezil sensing

For AChE inhibitor assay, different concentrations of donepezil (20 μL) was mixed with 50 μL of AChE (1.5 mU mL^{-1}), and then incubated for 20 min at 37 °C. Subsequently, ATCh (5.0 μM, 20 μL) and Cu^{2+} (100 nM, 5 μL) were added and reacted for another 30 min at 37 °C. Cu^{2+}-specific DNAzyme (10 nM, 10 μL) was added and incubated for 60 min at 37 °C to cleave the substrate strand. Then, a mixture that containing 100 nM H1 and 100 nM H2 was added and the HCR process was triggered. Finally, the AuNPs-DNA probe (4 nM, 100 μL) were introduced to recognize the HCR products. After incubating for 8 h at room temperature, the mixed solutions were transferred separately into a 1 cm quartz cuvette and the absorption spectra were measured. For the rat blood donepezil assay in vitro, each serum samples was mixed with different concentrations of donepezil (1.0 μM and 2.0 μM). After 30 min of incubation at 37 °C, the samples were diluted 10,000 times and the activity of AChE were detected. For the rat blood donepezil assay in vivo, after 4 h intragastrical administration, the obtained serum samples were diluted 10,000 times and the AChE activities were assayed. All the experimental conditions were identical to those described above.

3. Results and discussion

3.1. The principle of colorimetric sensing strategy

To date, many isothermal nucleic acid amplification techniques based sensing systems have been developed for various targets. However, their feasibility for AChE activity assay has not been explored. Herein, the colorimetric sensing strategy for AChE activity assay is put forward, which is based upon dual amplification of Cu^{2+}-specific DNAzyme and HCR by using AuNPs-DNA probe. As shown in Scheme 1A, in the absence of AChE, Cu^{2+} can specifically cleave the substrate strand of DNAzyme, then the HCR process cannot be triggered. Thus, the hairpin auxiliary probes H1/H2 can hybridize with AuNPs-DNA probe, which make them keep highly dispersed state. With the addition of AChE, its active substrate acetylthiocholine (ATCh) can be hydrolyzed to thiocholine and acetate acid. On the basis of the preferential binding affinity of thiol