Biosensors and Bioelectronics 78 (2016) 206–212

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Selective amyloid β oligomer assay based on abasic site-containing molecular beacon and enzyme-free amplification

Linling Zhu, Junying Zhang, Fengyang Wang, Ya Wang, Linlin Lu, Chongchong Feng, Zhiai Xu *, Wen Zhang *

School of Chemistry and Molecular Engineering, East China Normal University, Shanghai 200241, PR China

ARTICLE INFO

Article history:
Received 1 September 2015
Received in revised form
31 October 2015
Accepted 15 November 2015
Available online 17 November 2015

Keywords:
Amyloid β
Oligomer
Aptamer
Abasic site
Molecular beacon

ABSTRACT

Amyloid-beta (Aβ) oligomers are highly toxic species in the process of Aβ aggregation and are regarded as potent therapeutic targets and diagnostic markers for Alzheimer's disease (AD). Herein, a label-free molecular beacon (MB) system integrated with enzyme-free amplification strategy was developed for simple and highly selective assay of Aβ oligomers. The MB system was constructed with abasic site (AP site)-containing stem-loop DNA and a fluorescent ligand 2-amino-5,6,7-trimethyl-1,8-naphyridine (ATMND), of which the fluorescence was quenched upon binding to the AP site in DNA stem. Enzyme-free amplification was realized by target-triggered continuous opening of two delicately designed MBs (MB1 and MB2). Target DNA hybridization with MB1 and then MB2 resulted in the release of two ATMND molecules in one binding event. Subsequent target recycling could greatly amplify the detection sensitivity due to the greatly enhanced turn-on emission of ATMND fluorescence. Combining with Aβ oligomers aptamers, the strategy was applied to analyze Aβ oligomers and the results showed that it could quantify Aβ oligomers with high selectivity and monitor the Aβ aggregation process. This novel method may be conducive to improve the diagnosis and pathogenic study of Alzheimer's disease.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder, which can lead to loss of cognitive and behavioral function. The increasing incidence of AD-caused dementia and death worldwide prompts the researchers to focus on the early diagnosis and drug development for AD (Brookmeyer et al., 2007). Although the etiology of the disease is not clearly understood, compelling evidence shows that one of the characteristic pathological features of AD is the extracellular amyloid deposition in the brain (Selkoe, 2001; Hardy and Selkoe, 2002). The principal constituent of amyloid deposition is peptides of 40–42 amino acid called beta-amyloid (Aβ) derived from its transmembrane precursor protein (Kang et al., 1987). Previously, it was believed that neurotoxicity of Aβ protein could be mostly attributed to its fibrillar forms (Hardy and Higgins, 1992). However, recent studies have suggested that small, soluble Aβ oligomers rather than low molecular weight Aβ species or Aβ fibrils are major toxic species, which could cause physical degeneration of synapses and memory failure by strong synaptic binding (Gong et al., 2003; Kayed et al., 2003; Wolfe, 2002; Um et al., 2012). With injection of Aβ oligomer-selective antibody, recovery of memory in mice was observed (Takamura et al., 2011). Therefore, Aβ oligomers are potent diagnostic markers and therapeutic targets for AD (Salvadores et al., 2014). It is of great importance to develop a simple and powerful method to detect Aβ oligomers for the early diagnosis of AD and the screening of drugs for AD treatment. In particular, the selective assay of Aβ oligomers is a challenging subject due to the coexistence of Aβ monomers and fibrils in samples.

In the limited number of methods developed for specific Aβ oligomers detection, antibody or single-chain antibody fragment is one of the most commonly used tools (Wang et al., 2009; Veloso et al., 2014; Lambert et al., 2007). Based on antibody recognition, ELISA-type assays are frequently developed for detection of Aβ oligomers in patient fluids (Bruggink et al., 2013; Xia et al., 2009). Compared to antibodies, aptamers, which are DNA or RNA sequences, own the advantages of easy preparation, chemical stability and design versatility (Song et al., 2008; Iliuk et al., 2011; Fang and Tan, 2010). Fortunately, aptamers with high affinity and specifity for oligomeric amyloid proteins were selected (Tsukakoshi et al., 2012). Furthermore, using aptamer as recognition element can combine the recognition process with the versatile DNA molecular tools such as molecular beacons (MBs) (Tyagi and Kramer, 1996; Hamaguchi et al., 2001; Li et al., 2014). However, conventional MBs together with new kinds of designs require

* Corresponding authors.
E-mail addresses: zaxu@chem.ecnu.edu.cn (Z. Xu), wzhang@chem.ecnu.edu.cn (W. Zhang).

http://dx.doi.org/10.1016/j.bios.2015.11.048
0956-5663/

covalent modification of the two termini of DNA sequences, making the assay expensive and time-consuming. Therefore, quencher-free MBs with only one fluorophore labeled or label-free MB systems are highly desired (Hwang et al., 2004; Venkatesan et al., 2008; Sato et al., 2011). In addition, to fulfill the requirement of low-abundance nucleic acid and other bimolecular assay, MB probes have been associated with various signal amplification techniques. The frequently applied approaches are enzyme-assisted amplification methods of which polymerases, ligases, exonucleases or endonucleases were used to amplify the target recognition (Zhang et al., 2014; Gerasimova and Kolpashchikov, 2014; Li et al., 2008; Connolly and Trau, 2010; Ding et al., 2010; Wabuyele et al., 2003). These methods are playing increasing important roles in routine tests, while they are obsessed with complicated operation and high costs due to the participation of enzymes. To overcome these limitations, a number of enzyme-free amplification strategies have been developed including hybridization chain reaction and target recycling based on strand displacement (Wang et al., 2011; Huang et al., 2012; Qing et al., 2014; Zhu et al., 2013). These methods are effective and promising in amplified detection.

In this work, a selective assay for Aβ oligomer based on label-free abasic site (AP site)-containing MB system and enzyme-free amplification is successfully developed. An AP site was incorporated into the stem of hairpin-like DNA, and the specific binding of fluorescent ligand 2-amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) resulted in the quenching of ATMND fluorescence. The presence of target DNA can open one MB and sequentially the second different MB, leading to the release of target with two ATMND molecules. Target recycling on the basis of toehold-mediated strand displacement significantly amplified the sensitivity of the assay. Combining with aptamer, Aβ oligomers were successfully detected and the aggregation process from monomer to oligomer and fibril was monitored by this approach. The method avoids using expensive protein enzymes and complex modifications of molecular beacon, and does not require time-consuming thermal-cycling procedures. Therefore, this assay possesses excellent performance in DNA and protein detections and has the potential to advance the diagnosis of Alzheimer's disease.

2. Experimental section

2.1. Materials and chemicals

DNA oligonucleotides were obtained from Sangon Biotechnology Co. (Shanghai, China). The sequences are listed in Table 1. 2-Amino-5,6,7-trimethyl-1,8-naphthyridine (ATMND) was purchased from Enamine Ltd (Ukraine). Amyloid β peptide 1–40 was obtained from American Peptide Company (Sunnyvale, CA, USA). Other reagents were obtained from Sinopharm Chemical Reagent (Shanghai, China). All aqueous solutions were prepared using ultrapure water purified by Hitech laboratory water purification system (18.2 MΩ cm).

Table 1
The DNA sequences used in the experiments.

Name	Sequences (5′–3′)
MB1	5′-AAGTA GTGAT TGAGC CTGAT GAATG TCACT ACTTG AACTC GCATT CATCA **X**GCTC AATCA C-3′
MB2	5′-TGATG AATGC GAGTT CAAGT AGTGA CATTC ATCAG GCTCA ATCAC TACTT **X**AACT CGCAT T-3′
Tar	5′-ATTCA TCAGG CTCAA TCACT ACTT-3′
Tar-m1	5′-ATTCA TCAGG CTCGA TCACT ACTT-3′
Tar-random	5′-CAGCA CCATG CTCGT CTACT ACAA-3′
MB1$_{Aβ}$	5′-CGCAC CCGCC CCAAC ACCAC AGGCA GCGGG TGCGG AACTC GTGCC TGTGG T**X**TTG GGGC-3′
MB2$_{Aβ}$	5′-CCACA GGCAC GAGTT CCGCA CCCGC TGCCT GTGGT GTTGG GGCGG GTGCG **X**AACT CGTG-3′
Tar $_{Aβ}$	5′-GCTGC CTGTG GTGTT GGGGC GGGTG CG-3′

*X denotes the AP site having a propyl residue (Spacer C3)

2.2. Apparatus

Fluorescence spectra were measured at 10 °C with a Cary Eclipse spectrofluorophotometer equipped with a thermoelectrically temperature-controlled cell holder (Agilent Technologies, Palo Alto, CA, USA). The fluorescence spectra of ATMND were excited at 358 nm. The concentration of DNA was measured by a Cary 60 UV–vis spectrophotometer (Agilent Technologies, Palo Alto, CA, USA).

2.3. Preparation of Aβ oligomers and fibrils

The obtained Aβ peptide powder was first dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) at a concentration of 5 mg ml^{-1} and stored at −20 °C until use. For Aβ oligomers preparation, Aβ peptides were dissolved in 10 mM Tris–HCl (pH=7.4) and incubated at 37 °C for 10 h with shaking to form oligomers. Aβ fibrils were obtained by incubating Aβ monomers in 10 mM Tris–HCl (pH=7.4) with shaking at 37 °C for 48 h.

2.4. Construction of platform for amplification detection

To construct AP site-containing hairpins, MB1, MB2, MB1$_{Aβ}$, MB2$_{Aβ}$ were treated with a process of heating to 95 °C for 5 min and then gradually cooling down to room temperature for at least two hours. The hairpin probes were stored at 4 °C for later use. For a typical oligonucleotides sensing experiment, 10 μl of target DNA Tar, 10 μl of MB1 (0.75 μM) and 10 μl of MB2 (0.75 μM) were added sequentially into buffer (1 M NaCl, 0.05 M $MgCl_2$, 0.1 M PB, pH=7.4). The tube was placed in a water bath at 24 °C with shaking for 3 h. The solution was then allowed to be mixed with ATMND (0.5 μM). The final solution at a volume of 60 μl was kept at a constant temperature control at 4 °C. After 1 h, the fluorescence intensity was recorded. For Aβ monomers, oligomers or fibrils detection, different concentrations of Aβ samples were firstly mixed with 100 nM aptamer sequence of Tar$_{Aβ}$ for binding sufficiently. Then the cycling reaction was done as above.

3. Results and discussion

3.1. The principle of the label-free and enzyme-free amplified assay

The label-free and amplified assay was based on AP site-containing MB system integrated with enzyme-free target recycling by toehold-mediated strand displacement. As illustrated in Scheme 1, two DNA sequences of MB1 and MB2 with stem-loop structures were firstly designed with Spacer C3 AP site located in the stem. ATMND can bind to the AP site with high affinity by pseudo base pairing with cytosine opposite the AP site, resulting in its fluorescence quenching. In the presence of target DNA sequence, a toehold-mediated strand displacement was initiated by target DNA to open hairpin MB1, followed by ATMND liberation from MB1 since the AP site was left in a single-stranded state. Then, MB2 could hybridize to the newly exposed toehold of MB1, which not only recovered ATMND fluorescence again but also displaced target from MB1, accompanied by next target-triggered amplification. Under this dynamic assembly, enzyme-free amplification was easily realized and ATMND was circularly liberated by