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Short review

Electrochemical Methods for Detecting β-Amyloid Peptides and Monitoring Their Aggregation

Lin Liu[1,*], *Binbin Zhou*[1,2,*]

[1] College of Chemistry and Chemical Engineering, Anyang Normal University, Anyang, Henan 455000, People's Republic of China
[2] Hunan Institute of Food Quality Supervision Inspection and Research, Changsha, Hunan 410111, People's Republic of China
[*]E-mail: liulin82414@aliyun.com (L.L.); bbzhou1985@163.com (B.Z.)

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Amyloid-β (Aβ) peptides, the major constituent of the senile plaques in brain of Alzheimer's disease (AD) patient, have been regarded as the reliable molecular biomarkers and therapeutic targets for the diagnosis and prognosis of AD. The aggregation of Aβ is also believed to be a critical step in the aetiology of AD. Recently, electrochemical techniques have been successfully used to selectively detect kinds of Aβ species and monitor the oligomerisation and assembly of Aβ because of their high sensitivity, simplicity, rapid response, and compatibility with miniaturization. In this work, we summarized the progress in the development of electrochemical methods for detecting Aβ peptides and monitoring their aggregation.

Keywords: Alzheimer's disease; amyloid-β; electrochemistry; aggregation

1. INTRODUCTION

Alzheimer's disease (AD), the most common cause of dementia, is predicted to affect 1 in 85 people in the elderly by 2050. It is characterized by the presence of senile plaques in brain of AD patient, loss of memory and cognitive decline. The major constituent of the senile plaques is amyloid-β (Aβ) peptides containing 39–43 amino acid residues [1, 2]. Native Aβ peptides are the proteolytic cleavage products from amyloid precursor protein (APP) by β- and γ-secretase [3, 4]. They are composed of a hydrophilic fragment (first 16 amino acids from the N terminus) and a hydrophobic part (comprising 17–42 amino acids). The hydrophilic portion of Aβ contains three histidine residues (at 6th, 13th and 14th position) and a tyrosine residue (at 10th position), which are well known for their metal coordinating properties in various metalloenzymes. The hydrophobic amino acids 17–40 of Aβ

are responsible for aggregation and fibrillation. The general hypothesis is that Aβ peptides first coalesce to form small, soluble oligomers, followed by reorganization and assembly into long, insoluble, and often twisted, thread-like fibrils [5]. Recent observations suggested that soluble Aβ oligomers are capable of diffusing in the neuropil, potentially more neurotoxic than amyloid plaques, and likely responsible for the synaptic dysfunction and memory loss in AD patients and AD animal models [6, 7]. Several studies have also documented that the Aβ oligomers possess ligand-like properties, i.e., that they can bind to neurons with high affinity and specificity and trigger distinct cellular signal transduction responses, culminating in synaptic dysfunction and neuronal death [8-10]. Moreover, Aβ(1–40) (60–70%) and Aβ(1–42) (5–15%) are the two most abundant fractions of Aβ, while Aβ(1–42) exhibits greater tendency to form amyloid fibrils than Aβ(1–40). Recently, Aβ peptides have been regarded as the reliable molecular biomarkers and therapeutic targets for the diagnosis and prognosis of AD [11-13]. Thus, simple and sensitive analytical techniques able to selectively detect kinds of Aβ species and monitor the oligomerisation and assembly of Aβ have been developed, including surface plasmon resonance, localized surface plasmon resonance, electrochemistry and laser light scattering techniques [14]. Among them, electrochemical techniques are particularly popular due to their high sensitivity, simplicity, rapid response, and compatibility with miniaturization [15]. In this work, we summarized the progress in the development of electrochemical methods for detecting Aβ peptides and monitoring their aggregation.

2. ELECTROCHEMICAL DETECTION OF Aβ SPECIES

2.1 Aβ monomers

Peptides/proteins could be determined by electrochemical oxidation of their surface amino-acid residues including tyrosine, tryptophan, and cystine/cysteine [16]. It has been suggested that Aβ peptides can be determined via the intrinsic oxidation signal of tyrosine-10 (Tyr-10) residue in Aβ. For this consideration, Chikae et al. demonstrated that Aβ(1–40/1–42) peptides could be immobilized onto the saccharide layer formed by three steps: electrochemical deposition of AuNPs on a screen printed strip, SAMs formation of the acetylenyl group on AuNPs, and the cycloaddition reaction of an azide-terminated sialic acid to the acetylenyl group [17]. The captured Aβ(1–40/1–42) peptides were then detected by monitoring the intrinsic oxidation signal of tyrosine residue (0.6 V vs Ag/AgCl) with differential pulse voltammetry. In this study, they also found that the addition of other proteins such as bovine serum albumin and insuline presented no tyrosine current, indicating that the saccharide surface shows enough specificity to Aβ species. Also, Prabhulkar et al. developed a multiplexed, implantable immunosensor to quantify the Aβ(1–40/1–42) concentration ratio in CSF from mice using triple barrel carbon fiber microelectrodes [18]. Aβ(1–40) and Aβ(1–42) peptides were first selectively captured by the specific antibodies modified on the electrode surface and then determined by measuring the intrinsic oxidation signal of tyrosine residue in Aβ at 0.65 V (vs. Ag/AgCl). The detection ranges for Aβ(1–40) and Aβ(1–42) were found to be 20 ~ 50 nM and 20 ~ 140 nM, respectively. These methods

based on directly monitoring the intrinsic oxidation signal of tyrosine residue are simple but not enough sensitive to detect nanomolar Aβ in human brain.

Recently, our group developed two sensitive and selective electrochemical immunosensors for determining the total concentration of Aβ(1–40/1–42) and the relative level of Aβ(1–42) by signal amplification of Aβ(1–16)-heme-modified gold nanoparticles (AuNPs) (Aβ(1–16)-heme-AuNPs) and p-aminophenol (p-AP) redox cycling, respectively [11, 13, 19]. In the first work, monoclonal antibody (mAb) specific to the common N-terminus of Aβ was immobilized onto gold electrode for the capture of Aβ(1–16)–heme–AuNPs (Fig. 1) [13]. The captured Aβ(1–16)-heme-AuNPs facilitated the electrocatalytic O_2 reduction to H_2O_2. Pre-incubation of the mAb-covered electrode with native Aβ decreased the amount of Aβ(1–16)–heme–AuNPs immobilized onto the electrode, thus leading to the decrease of the reduction current of O_2. The currents were inversely proportional to the total Aβ concentrations in the range of 0.02 ~ 1.50 nM.

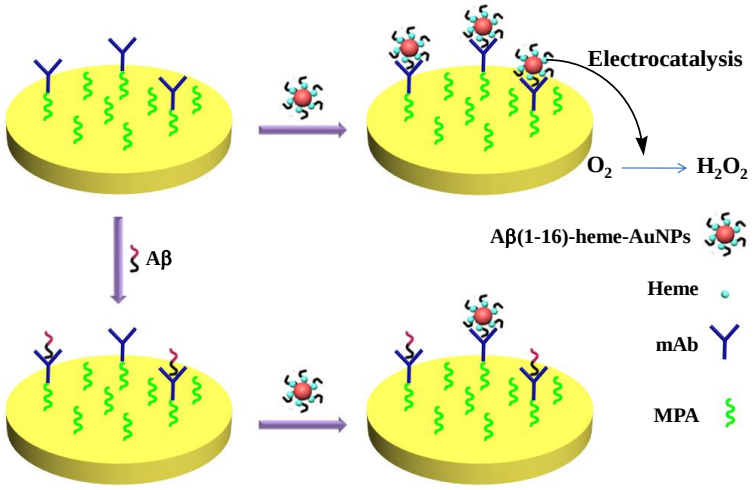

Figure 1. Schematic representation of Aβ detection. More Aβ(1–16) –heme–AuNPs are attached onto the mAb-covered electrode without the Aβ capture step (top). A smaller number of Aβ(1–16)–heme–AuNPs are attached after incubation of the electrode with Aβ species (bottom). Reprinted with permission from [13]. Copyright 2013 Elsevier.

However, since the levels of Aβ(1–42) or total Aβ may differ by gender and age, assays of Aβ(1–42) or total Aβ only might be unable to discriminate between AD and health control or other types of dementia. For this consideration, we presented another electrochemical immunosensor for the detection of both Aβ(1–42) and total Aβ using p-AP redox cycling (Fig. 2) [11]. Specifically, the conjugates performed between streptavidin-conjugated alkaline phosphatase (SA-ALP) and biotinylated Aβ peptides were captured by the antibodies-modified gold electrodes. The captured ALP promoted the production of electrochemically active p-AP from the p-aminophenylphosphate (p-APP) substrate. In the presence of tris(2-carboxyethyl)phosphine (TCEP), p-AP could be cycled after its electro-oxidization on the electrode, enabling the increase of the anodic current. Because of the competing interaction between the native Aβ species and the Aβ-biotin-SA-ALP conjugates to the pre-immobilized antibodies, the contents of the enzymatically produced p-AP in two separate

electrochemical cells were inversely proportional to the concentrations of Aβ(1–42) or total Aβ. The detection limit of 5 pM was a little lower than that (10 pM) obtained by the signal amplification of Aβ(1–16)–heme–AuNPs.

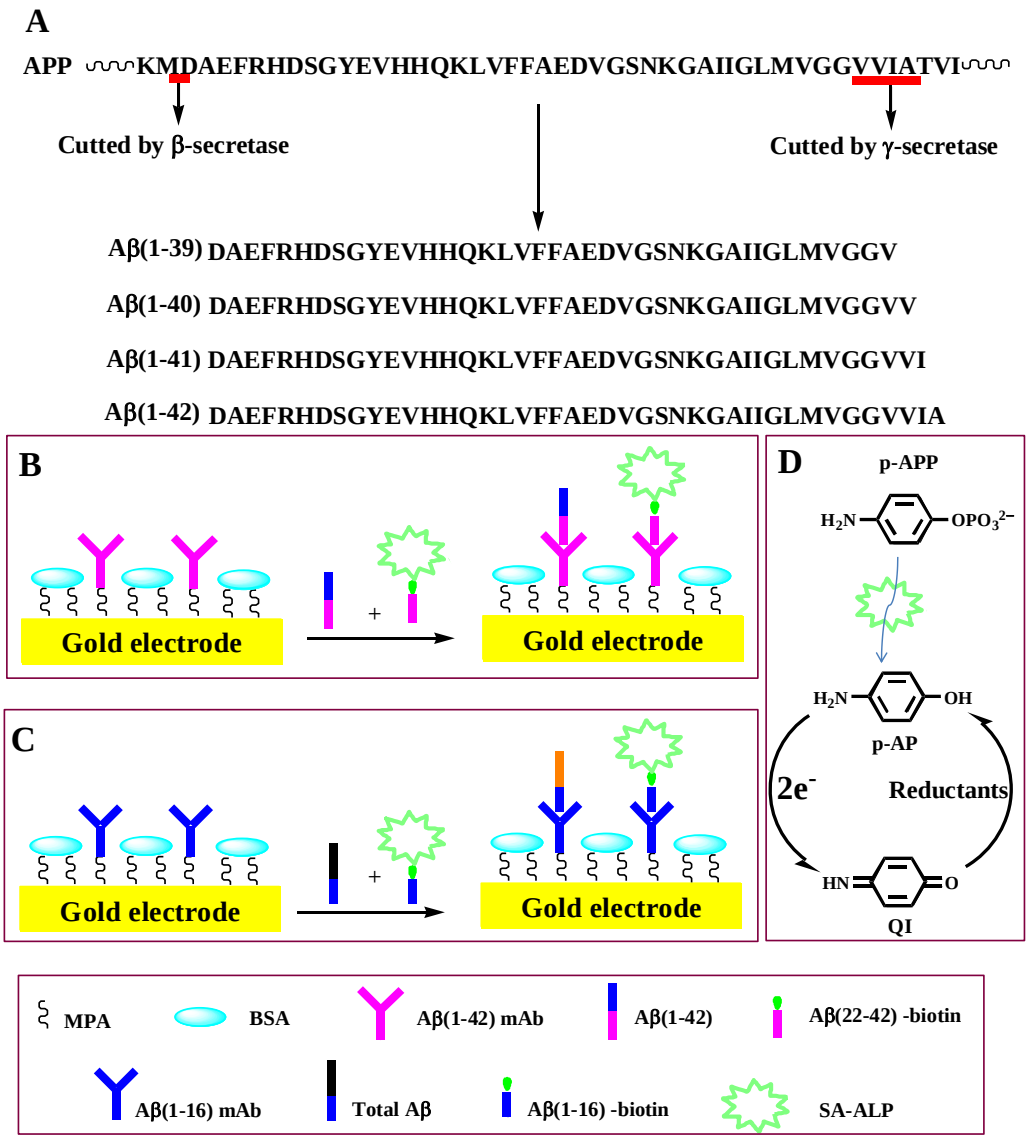

Figure 2. Sequence of native Aβ produced from APP (A) and schematic representation for the detection of Aβ(1-42) (B) and total Aβ (C) using p-aminophenol redox cycling by chemical reductants (D). Reprinted with permission from [11]. Copyright 2014 Elsevier.

Lately, Rama et al. reported a disposable competitive electrochemical immunosensor for the detection of Aβ(1–42) [20]. In the work, screen-printed carbon electrode nanostructured with AuNPs generated "in situ" was used as the transducer surface. Biotinylated Aβ(1–42) immobilized on the streptavidin-modified electrode surface through the streptavidin-biotin interaction allowed for the capture of anti-Aβ(1–42) antibody; then, alkaline phosphatase (ALP)-labeled anti-rabbit IgG antibody was anchored onto the sensing electrode. The electrochemical signal was carried out by the anodic

stripping of enzymatically generated silver by cyclic voltammetry. The competition binding between native Aβ(1–42) and biotinylated Aβ(1–42) pre-immobilized on the electrode limited the capture of anti-Aβ(1–42) antibody, thus preventing the attachment of ALP-labeled anti-rabbit IgG antibody and causing the decrease in the resulting current. With this method, a detection limit of 0.1 ng/mL for Aβ(1–42) was achieved.

Based on the uniform physical, chemical and biomedical properties of porous magnetic microspheres (PMMs) and the good electrocatalytic activity of AuNPs towards hydrogen evolution reaction (HER) [21], Merkoçi's group reported the magnetosandwich immunoassays of AD biomarkers (both Aβ and ApoE) in human samples [22]. Compared with the commercial magnetic particles, PMMs have two main advantageous properties: (1) the carboxyl groups in PMMs allow the covalent bonding of antibodies through EDC-mediated amine coupling reaction, and (2) the high porosity of PMMs offer large surface area which in turn increases quantity of the immobilized antibodies and enhances the catalytic activity of the captured AuNPs electrocatalytic tags.

It has been suggested that gelsolin binds to both Aβ(1–40) and Aβ(1–42) in a concentration-dependent manner [23]. For this consideration, Shi's group reported two "sandwich-like" electrochemical biosensors for the detection of Aβ(1–40/1–42) peptides in the CSF and various brain regions with gelsolin as the biorecognition element [24, 25]. Screen-printed carbon electrodes modified with multiwalled carbon nanotubes (MWCNTs) and AuNPs were employed for the immobilization of gelsolin and the follow-up capture of Aβ(1–40/1–42) (Fig. 3). In their first work, the gelsolin-Au-thionine bioconjugates were used to recognize the captured Aβ(1–40/1–42) species by the gelsolin-Aβ interaction [24].

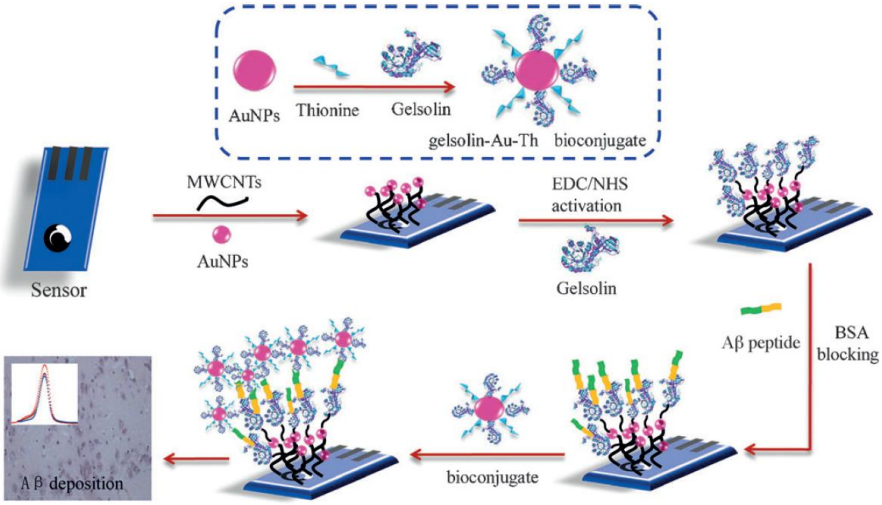

Figure 3. A schematic illustration of the electrochemical detection of Aβ(1–40/1–42) by using a gelsolin-Au-Th bioconjugate as a probe. Reprinted with permission from [24]. Copyright 2014 John Wiley and Sons.

The concentrations of Aβ(1–40/1–42) peptides were determined by monitoring the electrochemical reduction of thionine (Th). In the second work, the HRP–Au–gelsolin nanohybrid

prepared by one-pot modification of AuNPs with horseradish peroxidase (HRP) and gelsolin was employed as the nanoprobe for the recognition of the captured Aβ species [25]. The attached HRP then catalyzed the oxidization of 3,3′,5,5′-tetramethylbenzidine (TMB) substrate in the presence of H_2O_2, which produced a measurable electrochemical signal. The detection limits of these two methods were 50 pM and 28 pM, respectively. Differing from the previous strategies for Aβ detection, these methods obviates the use of antibodies for the capture and recognition of Aβ. At the same time,

2.2 Aβ oligomers

The principal species deposited within the parenchyma of the Alzheimer's disease brain is Aβ(1–42). In addition to monomeric Aβ(1–42) and total Aβ, one of clinical practices of Alzheimer's disease diagnostics at present is based on the detection of Aβ oligomers [14]. Several groups also described the specific detection of Aβ(1–42) oligomers with electrochemical techniques. For example, Li et al. demonstrated the detection of Aβ(1–42) oligomers on gold electrodes covered with ferrocene (Fc)-conjugated peptides (11-mercaptoundecanoic acid (MUA)-RGTWEGKWK-Fc) [26]. The capture of Aβ(1–42) oligomers caused the change of surface electron transfer and the tune of the frequency of square wave voltammetry (SWV). By fine-tuning the scan pulse frequency of SWV to synchronize with the surface electron transfer of the immobilized peptide probes, the biosensor allow for the detection of Aβ(1–42) oligomers at the concentration as low as 240 pM (equivalent monomer). Recently, Li et al. also reported a general way to assay proteins including Aβ(1–42) oligomers with the host–guest chemistry of cucurbituril (Fig. 4) [27]. Specifically, the electrochemical reporter methylviologen could be introduced onto the RGTFEGKF-MUA covered electrode surface to produce an electrochemical signal via the formation of a cucurbituril-methylviologen-RGTFEGKF supermolecule complex. The binding of Aβ (1–42) oligomers to RGTFEGKF inhibited the formation of the supermolecule and prevented the introduction of methylviologen on the electrode surface. As a result, the currents were inversely proportional to the levels of Aβ(1–42) oligomers. The detection limit of 48 pM was lower than that (240 pM) obtained by their first method.

Recently, cellular prion protein (PrP^C) has been identified in a genome-wide screen as a high-affinity receptor for Aβ oligomers; many studies have also indicated that the core region of PrP^C for the interaction between PrP^C and Aβ oligomers is PrP(95-110), located within the unstructured N-terminal region of PrP^C with an amino acid sequence of THSQWNKPSKPKTNMK [10, 28-34]. Based on this fact, Rushworth et al. suggested the specific detection of Aβ oligomers using THSQWNKPSKPKTNMK-modified polymer-functionalized gold screen-printed electrodes [35]. Binding of Aβ oligomers to the sensor surface induced the increase of the electrochemical impedance. With this method, Aβ oligomers could be distinguished with their monomeric and fibrillar species. The detection limit of this method was approximately 100 pM (equivalent monomer). Based on the specific interaction between Aβ oligomer and PrP(95-110) peptide, we presented an antibody-free electrochemical method for the detection of Aβ oligomer [12]. In this work, cysteine-containing PrP(95–110) was immobilized on a gold electrode to capture oligomeric Aβ(1–42); then, alkaline phosphatase (ALP)-conjugated PrP(95–110) was used for the recognition of the captured AβO and the

generation of redox species. To improve the detection sensitivity, the electrochemical signal was amplified with an enzymatic reaction plus an "outer-sphere to inner-sphere" electrochemical–chemical–chemical (ECC) redox cycling using ferrocene methanol as the redox mediator. As a result, a detection limit of 3 pM for equivalent monomer was achieved.

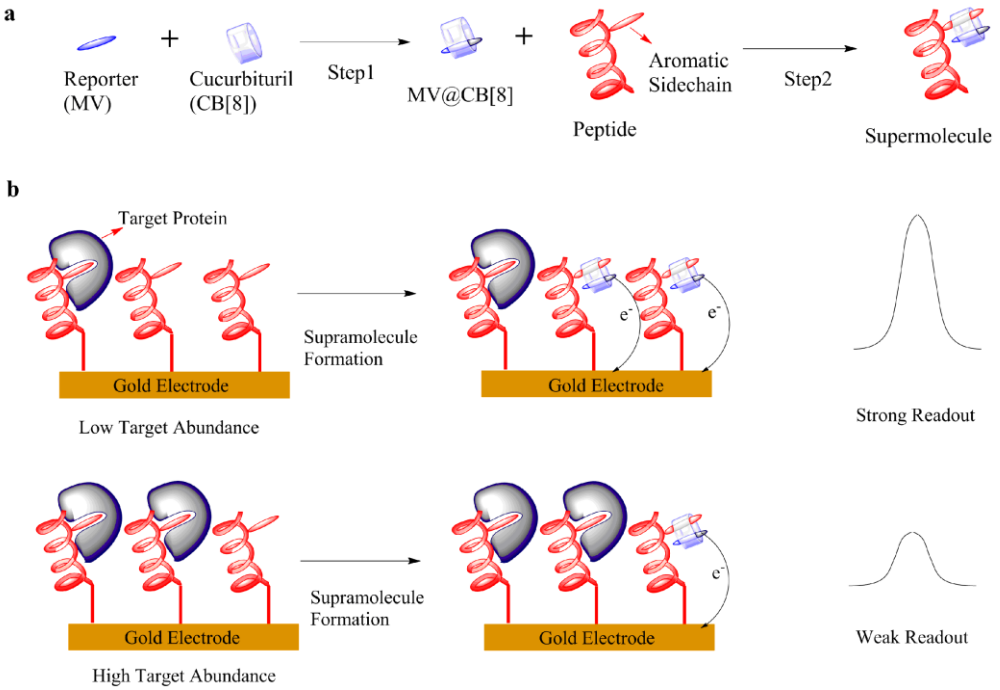

Figure 4. (a) Coupling peptide with reporter via supermolecule formation and (b) assay for protein detection. Reprinted with permission from [27]. Copyright 2013 American Chemical Society.

3. PROBING OF Aβ AGGREGATION

3.1 Electrochemical oxidation of tyrosine residue

Investigation of amyloidogenesis and formation dynamics of the prefibrillar intermediates (protofibrils, protofilaments or oligomers) are important to understand the routes of AD pathogenesis. Usually, Aβ aggregation can be monitored by circular dichroism spectroscopy, fluorescence spectroscopy, and atomic force microscopy (AFM). Particularly, probing of Aβ aggregation by thioflavin T (ThT) fluorescence assay has been believed to be a standard method. As talked above, Aβ could be determined by electrochemical oxidation of their surface tyrosine residue. Recently, electrochemical techniques have also been successfully used in probing of Aβ aggregation by measuring the intrinsic electroactivity of the tyrosine residue in Aβ. Typically, in 2005, Vestergaard and co-workers for the first time suggested the kinetic study of Aβ(1–40/1–42) fibrillization by performing square-wave voltammetry (SWV) to measure the electrochemical signal of tyrosine oxidation at a glass carbon electrode [36]. In this approach, tyrosine becomes enveloped during the self-assembly of Aβ monomers, leading to a decrease in its oxidation current over time.

Metal-induced Aβ aggregation may favor the production of reactive oxygen species and cause more damage to cells in brain [37, 38]. Based on the electrochemical oxidation of tyrosine residue, metal-induced Aβ aggregation could also be monitored. For example, Geng et al. studied the aggregation kinetics of Aβ in the absence or presence of Zn and Cu in 2008 [39]; Huang et al. suggested that Zn and Fe increased Aβ aggregation rate by binding to the metal chelating histidine-rich region of Aβ [40]. Moreover, polyphenols are a class of strong antioxidants and metal chelators, with characteristics that are of beneficial therapeutic values for their development as candidates targeting neurodegenerative and metal-induced diseases. Zhang et al. examined the interaction of (–)-epigallocatechin-3-gallate (EGCG) with Aβ(1–40) and Cu(II) based on the electrochemical oxidation of the tyrosine-10 residue [41].

At present, a critical target for developing AD drug therapies is the inhibition and/or disassembly of misfolded β-sheets. Thus, the discovery of generic inhibitors in order to control the formation of amyloid fibrils and early oligomers is highly desired. Recently, several studies have demonstrated the applicability of electrochemical analysis for evaluating the interaction between Aβ aggregates and their inhibitors in vitro [42-44]. For example, β-Sheet breaker (BSB) peptides are a class of inhibitors that specifically bind to Aβ peptide for preventing and reversing its conversion to a β-sheet-rich aggregated structure; Veloso et al. investigated the amyloid formation in the presence of a β-sheet breaker pentapeptide LPFFD by following the oxidation of tyrosine-10 residue in Aβ using square-wave voltammetry [43].

3.2 Voltammetric characterization of electroactive modulators of Aβ aggregation

Although the intercalative properties of amyloid β-sheet with ThT or its derivatives have been well-documented by fluorescence, their electrochemical study is less reported. Recently, Veloso et al. investigated the interfacial properties and the course of Aβ(1–40/1–42) aggregation in the presence of benzothiazole dyes (both ThT and itsderivative BTA-1) by electrochemistry. This work presented a new and simple tool for probing of amyloid formation based on the electrochemical oxidation signal of the benzothiazole dye [45]. Electrochemical techniques were then used to determine the progressive changes in amyloid conformation by time-resolved voltammetric characterization of electroactive modulators of Aβ aggregation [46, 47]. For example, congo red (CR) is a diazo dye commonly used for the staining of amyloid plaques in post-mortem tissue; Veloso also reported the first electrochemical investigation of the interaction between CR and Aβ by monitoring the anodic peak current intensity of CR with incubation time [46]. Specifically, incorporation of the aromatic rings of CR into the highly hydrophobic oligomers led to a decrease in the amount of free CR available to the surface of screen-printed electrode and a subsequent decline in the oxidation current of CR. Moreover, Veloso et al. investigated the voltammetry of two new sym-triazine derivatives (TAE-1, TAE-2) as modulators of Aβ aggregation, and found that the dissociation of TAE from Aβ(1–42) induced progressive shifts in their peak potential and anodic peak current [47]. These two works indicated that electrochemistry cloud offer a simple, cost-effective and high-throughput tool to accelerate the discovery of novel anti-Aβ compounds and identify drugs exhibiting high Aβ association.

Moreover, it has been suggested that peptide sequence of KLVFF is critical for fibril formation and has the potential to disrupt aggregation and even causes dissolution of mature Aβ fibrils. To probe disruption of Aβ aggregation, Beheshti et al. prepared ferrocene-peptide conjugates (denoted as Fc-KLVFF) for the first time and studied its interaction with Aβ(12–28) [48]. The results demonstrated that the inhibitory potency of the Fc-peptide conjugates influenced Aβ(12–28) aggregation, coinciding with their ability to interact with the Aβ(12–28) surface.

3.3 Electrochemical impedance spectroscopy

Electrochemical impedance spectroscopy (EIS) has been used extensively to examine the property of self-assembled monolayers. Also, EIS has been shown to be a promising method for the analysis of Aβ aggregation in the presence of small molecule inhibitors and accelerators. For example, Partovi-Nia studied the interactions of Aβ(12-28)-Cys immobilized on a gold surface with CR and BSB peptide. The various aspects of the peptide film have been examined using different electrochemical and surface analytical techniques. The results of electrochemical impedance spectroscopy in $[Fe(CN)_6]^{3-/4-}$ demonstrated that CR and BSB have different influences on the electrochemical property of the Aβ(12-28)-Cys film. In the case of CR, the electrochemical resistance of Aβ(12-28)-Cys-covered electrode decreased significantly presumably due to the better penetration of $[Fe(CN)_6]^{3-/4-}$ into the film. However, in the case of BSB, the resistance increased, which is probably attributed to the interaction of BSB with the Aβ(12-28)-Cys on the surface and the resulting formation of a film that presented a higher resistance for electron transfer of $[Fe(CN)_6]^{3-/4-}$ [49]. Furthermore, Huang et al. studied the interaction of resveratrol (a polyphenolic compound) and Aβ using EIS. In this report, incubation of the oligomer-modified gold electrode with free Aβ in solution caused the increase in the charge transfer resistance due to the growth of Aβ fibrils on electrode surface. In contrast, the presence of resveratrol significantly slowed down the formation of Aβ aggregates and prevented the growth of Aβ fibrils, thus inhibiting the increase in the charge transfer resistance [50].

Very recently, Veloso et al. developed a novel electrochemical immunosensor to monitor the change in distribution of oligomers and fibrils of Aβ during stimulated aggregation using EIS. As shown in Fig. 5, conformation-specific Aβ antibodies of A11 and OC were immobilized onto gold compact disc electrodes to selectively capture oligomers and fibrils, respectively. The degree of surface binding was subsequently determined by measuring the charge-transfer resistance of $[Fe(CN)_6]^{3-/4-}$ probes. After Aβ oligomers were structurally remodeled by the aggregation disrupting agents (e.g.TAE-1 and TAE-2), they showed reduced quantities of the A11-reactive epitopes and the lower toxicities. Thus, the effect of TAE-1 and TAE-2 on the distribution of fibrillar and toxic oligomeric species of Aβ(1−42) could be evaluated by electrochemical impedance spectroscopy [51].

Moreover, Zhao et al. presented a simple and novel electrochemical method for probing of the interaction of Aβ and bilayer lipid membrane [52]. Specifically, the bilayer lipid membrane modified electrode induced a significant steric hindrance to prohibit $[Fe(CN)_6]^{3-/4-}$ approaching to the electrode. However, after pre-incubation of the modified electrode with Aβ peptides, a noticeable

electrochemical response of $[Fe(CN)_6]^{3-/4-}$ can be obtained on the lipid membrane modified electrode due to the formation of ion channels in the lipid membrane. Further experimental results indicated that the peak currents of $[Fe(CN)_6]^{3-/4-}$ increased with increase of Aβ concentrations and incubation time. Meanwhile, the increase was found to be prevented by a well-known inhibitor (EGCG) of Aβ aggregation. This work is valuable for revealing the Aβ toxicity and screening of its aggregation inhibitors.

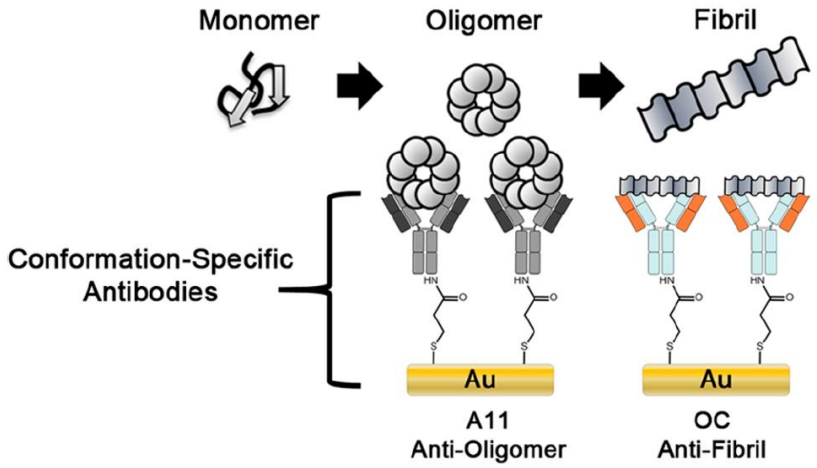

Figure 5. Detection principle for monitoring Aβ(1−42) fibrils and toxic oligomers using conformation specific antibodies in conjunction with EIS. Reprinted with permission from ref. [51]. Copyright 2014 American Chemical Society.

4. CONCLUSION

In summary, we summarized the recent progress in detection of Aβ monomers and oligomers and probing of Aβ aggregation with electrochemical techniques. Since the small oligomeric assemblies of Aβ, especially Aβ(1–42), are more toxic than their monomeric and fibrillar species, assay of the relative level of Aβ(1–42) monomers or oligomers will be more precise for diagnosis and prognosis of AD. Moreover, antibody-free electrochemical biosensors provide simple and cost-effective detection procedures for both monomeric and oligomeric Aβ species. Multiplexed detection of different samples could be conveniently performed with multichannel electrochemical cells with micro-fabricated and nanofabricated working electrodes; thus, more reliable information would be gleaned from the high-throughput assays. The reported electrochemical techniques for monitoring Aβ aggregation can also provide simple, rapid and sensitive tools to accelerate the discovery of novel anti-Aβ compounds and identify drugs exhibiting high Aβ association.

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