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Short communication

Competitive electrochemical immunoassay for detection of β-amyloid (1–42) and total β-amyloid peptides using p-aminophenol redox cycling

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ABSTRACT

β-Amyloid (1–42) peptide (Aβ(1–42)) is believed to be important for diagnosis and prognosis of Alzheimer's disease (AD) serving as a reliable molecular biomarker. However, the levels of Aβ(1–42) may differ by gender and age; thus, assay of Aβ(1–42) only might be unable to discriminate between AD and health or other types of dementia. In this work, we reported a sensitive and selective electrochemical method for detection of both Aβ(1–42) and total Aβ using p-aminophenol (p-AP) redox cycling on antibody-modified gold electrodes. Specifically, the conjugates performed between streptavidin-conjugated alkaline phosphatase (SA-ALP) and biotinylated Aβ peptides were captured by the antibody-modified electrodes, which induced the production of electrochemically active p-AP from the p-aminophenyl phosphate (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 native Aβ competed with the conjugates to bind the anchored antibody, the signal decreased with the increase of native Aβ concentration. A detection limit of 5 pM was achieved. To demonstrate the viability of the method for analysis of Aβ(1–42) and total Aβ in real sample, artificial cerebrospinal fluid (aCSF) containing Aβ(1–40), Aβ(1–42) and Aβ(1–16) was tested. We believe that the simultaneous detection of Aβ(1–42) and total Aβ would be valuable for the early diagnosis of AD.

1. Introduction

Alzheimer's disease (AD) is the most common chronic and progressive form of neurodegenerative disease, which is characterized by gradual loss of cognitive function and synaptic integrity, selective neuronal death and abnormal formation of neurotic and core plaques in the cerebral cortex (Hardy and Selkoe, 2002). AD is predicted to affect 1 in 85 people globally by 2050, but there is no effective cure for the disease to date (Brookmeyer et al., 2007; Rolinski et al., 2010). One of the pathological hallmarks of AD is the deposition of β-amyloid peptides (Aβ) in the brain. The peptides, including 39–42 amino acid residues, are proteolytic cleavage product from amyloid precursor protein (APP) by β- and γ-secretase (Fig. 1A) (Geng et al., 2011; Hardy and Selkoe, 2002). Among the various Aβ species in human cerebrospinal fluid (CSF), Aβ(1–40) (60∼70%) and Aβ(1–42) (5∼15%) are the two predominant cleavage products, while Aβ(1–42) exhibits greater tendency to form amyloid fibrils than Aβ(1–40) (Ogi et al., 2013; Stravalaci et al., 2011; Vestergaard et al. 2005). The level of Aβ(1–42) found in an AD brain is lower than that in a control due to its aggregation (Golde et al., 2000). With regard to the close relationship between AD development and Aβ(1–42) level, Aβ(1–42) is considered a promising biomarker for AD (Golde et al., 2000; Kang et al., 2009; Wang et al., 2011; Wang et al., 2012; Xia et al., 2010). Therefore, current clinical practice of AD diagnostics is based on the detection of Aβ(1–42) with enzyme-linked immunosorbent assay (ELISA). The reported ELISA for Aβ detection is reliable but labor intensive, and requires the use of relatively expensive enzyme-linked antibody for Aβ recognition and carcinogenic substrate for chemiluminescent detection; in addition, such procedure is not suitable for early diagnostics of AD (Mustafa et al., 2010). Recently, a few new methods have been developed to detect Aβ(1–42), such as capillary electrophoresis, resonance light scattering, surface plasmon resonance (SPR) and gold nanoparticle-based dot-blot immunoassay (Golde et al., 2000; Picou et al., 2010; Wang et al., 2011; Wang et al., 2012; Xia et al., 2010). However, these methods are usually expensive, require complicated instruments and/or lack sensitivity. Moreover, the levels

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of Aβ(1–42) may differ by gender and age; thus, an assay of Aβ(1–42) might be unable to discriminate between AD and health or other types of dementia (Doong et al., 2010; Golde et al., 2000; Southwick et al., 1996; Xia et al., 2010). The goal of the present work is to develop an electrochemical method for simultaneous detection of Aβ (1–42) and total Aβ in view of the high sensitivity, simplicity and rapid response of electrochemical biosensors (Chen et al., 2012; Liu et al., 2013b; Venkatanarayanan et al., 2013; Yasukawa et al., 2012; Zhuang et al., 2012).

At present, the increasing demand for developing electrochemical biosensors is driving the enhancement of detection sensitivity by selecting different signal amplification strategies (Yasukawa et al., 2012). Thus, many attempts have been made to reduce the detection limit by amplifying the signal using various labels, such as functionalized liposomes, enzymes, carbon nanotubes and nanoparticles (Das et al. 2006; Moreno-Guzmán et al., 2012; Ojedaa et al., 2012; Patolsky et al., 2000; Xia et al., 2013a). A more recent strategy for signal amplification is to employ an approach that entails multiple signal amplification such as enzymatic reaction plus redox-cycling reaction (Jiang et al., 2012; Yang, 2012). For example, alkaline phosphatase (ALP) is one of the most used enzymatic labels for design of absorption biosensors; Yang's group reported a series of electrochemical immunosensors with ALP-based p-aminophenol (p-AP) redox cycling by chemical reducing reagents on the indium-tin oxide (ITO) electrodes (Akanda et al., 2012; Das et al., 2007; Kim et al., 2003; Kwon et al., 2006; Walter et al., 2011). In this process, ALP dephosphorylates p-aminophenyl phosphate (p-APP) enzymatically to produce electroactive species p-AP, which is oxidized electrochemically to p-quinone imine (QI). Immediately QI is reduced by reducing reagent to regenerate p-AP, which greatly enhances the anodic current of p-AP (Fig. 1D). Recently, we compared the performances of p-AP redox cycling by different reducing reagents and reported the detection of microRNA on gold electrodes covered with 6-mercapto-1-hexanol/DNA self-assembled monolayers (SAMs) (Xia et al., 2013b). In the present work, we developed a sensitive and selective electrochemical immunosensor for detection of both Aβ(1–42) and total Aβ using p-AP redox cycling by tris(2-carboxyethyl)phosphine (TCEP).

2. Experimental

2.1. Chemicals and reagents

3-Mercaptopropionic acid (MPA), 1-ethyl-3-[3-dimethylaminopropyl]carbodiimide hydrochloride (EDC), N-hydroxysulfosuccinimide (NHSS), ethanolamine (EA), TCEP, nicotinamide adenine dinucleotide (NADH), cysteamine hydrochloride, streptavidin-conjugated alkaline phosphatase (SA-ALP) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich. p-Aminophenylphosphate (p-APP) was

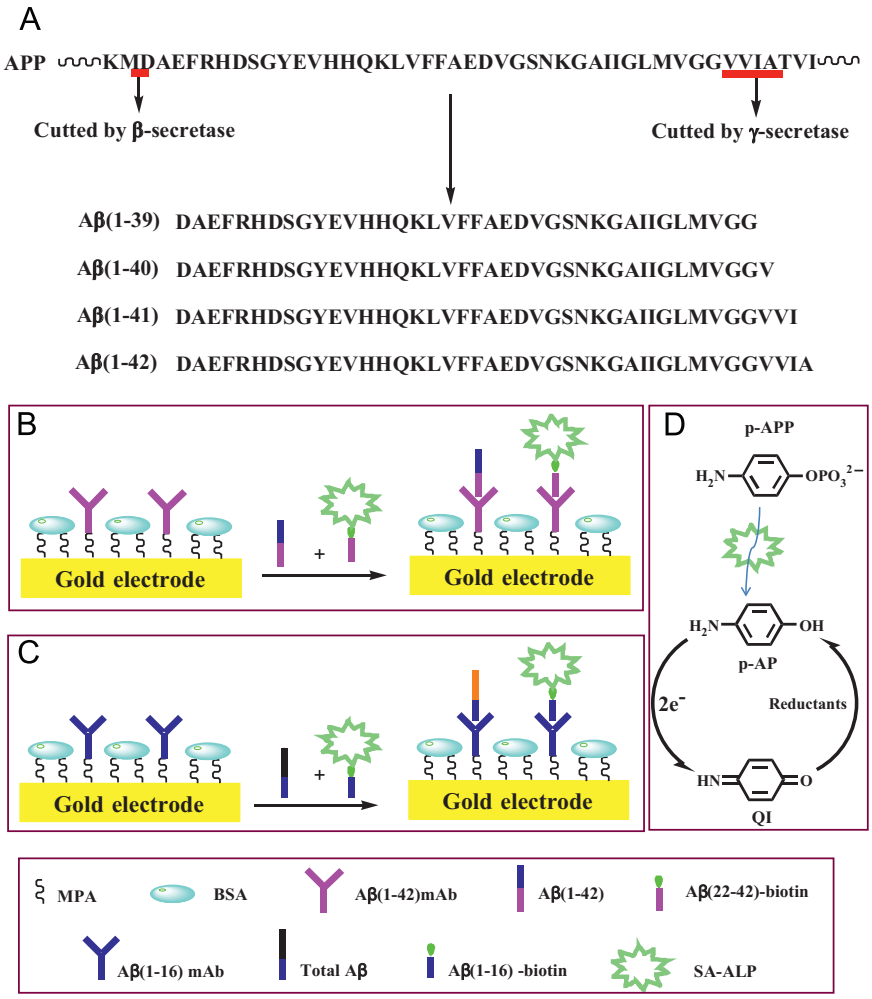

Fig. 1. Sequence of native Aβ produced from APP (A) and schematic representation for the detection of Aβ(1–42) (B) and total Aβ (C) using p-aminophenol redox cycling by chemical reductants (D).

obtained from Enzo Life Sciences, Inc. (Farmingdale, NY, USA). Purified monoclonal antibody (mAb) of Aβ(1–16) (clone 6E10) specific to the common N-terminus of Aβ and Aβ(1–42) mAb (clone 12F4) specific to the C-terminus of Aβ(1–42) were provided by Covance Inc. (Dedham, MA, USA). Aβ and biotinylated peptides were synthesized and purified by ChinaPeptides Co., Ltd (Shanghai, China). The sequences of biotinylated peptides are Asp–Ala–Glu–Phe–Arg–His–Asp–Ser–Gly–Tyr–Glu–Val–His–His–Gln–Lys–biotin (denoted as Aβ(1–16)-biotin) and biotin–Glu–Asp–Val–Gly–Ser–Asn–Lys–Gly–Ala–Ile–Ile–Gly–Leu–Met–Val–Gly–Gly–Val–Val–Ile–Ala (denoted as Aβ(22–42)-biotin). The conjugates of Aβ(1–16)-biotin–SA–ALP and Aβ(22–42)–biotin–SA–ALP are prepared by mixing biotinylated Aβ peptides and excess concentration of SA–ALP (1 μM) through the strong streptavidin–biotin interaction. Biotinylated peptides (0.1 mM) were dissolved with 10 mM NaOH and diluted to the desired concentration with phosphate-buffered saline solution (PBS buffer, 50 mM, pH 7.4) before use. Artificial cerebrospinal fluid (aCSF) including 150 mM NaCl, 3,0 mM KCl, 1.4 mM $CaCl_2 \cdot 2\,H_2O$, 0.8 mM $MgCl_2 \cdot 6\,H_2O$, 1 mM phosphate was prepared in-house (Hegnerová et al., 2009). The Aβ stock solutions (0.1 mM) were prepared freshly as in our previous study (Liu et al., 2011; Xia et al., 2010). The stock peptide concentrations were determined using a Cary 50 spectrophotometer with the extinction coefficient (ε) of 1410 $M^{-1}\,cm^{-1}$ at 276 nm.

2.2. Performances of p-AP redox cycling on MPA-covered gold electrode

Gold disk electrodes with a diameter of 2 mm were polished with diamond pastes down to 3 μm and alumina pastes down to 0.05 μm, and then sonicated in ethanol and water. The MPA-covered electrodes were prepared by immersing the cleaned gold electrodes in an ethanol solution containing 10 mM MPA in the darkness for 12 h. The performances of p-AP redox cycling by different reducing reagents on the modified electrodes were evaluated in the PBS solution containing 50 mM Na_2SO_4. Voltammetric determination was carried out on a DY2013 electrochemical work station (Digi-Ivy, Inc., Austin, TX).

2.3. Immobilization of antibody

The immobilization method of antibody is important for fabrication of affinity biosensor because it governs the stability and applicability of the developing system. In this work, mAb was immobilized onto gold electrode by cross-linking mAb molecules onto the MPA SAMs surface via the EDC/NHSS-mediated amine coupling reaction. Such immobilization model facilitates the binding of Aβ to mAb (Ammar et al., 2013; Haes et al., 2005; Wang et al., 2012). Briefly, MPA-covered electrodes were soaked in a solution comprised of 0.2 M EDC and 0.1 M NHSS for 15 min (Liu et al., 2013a), washed with water and soaked in a 1-μM mAb solution for 12 h at 4 °C. This step was followed by casting 0.1 mM EA containing 10-μM BSA onto the electrode for 30 min to block the unreacted sites.

2.4. Detection of Aβ

For the assay, a 10-μL mixture comprising the conjugate of a fixed concentration and Aβ of varying concentrations was cast onto the mAb-covered electrode for 20 min. After washing with water to remove the excess physiadsorbed SA-ALP and drying with nitrogen, the electrode was immersed in a 50-μL PBS solution containing 0.5 mM p-APP and 2 mM TCEP for 60 min in a home-made plastic electrochemical cell. Then, voltammetric and amperometric detection of the produced p-AP was carried out with a platinum wire and a Ag/AgCl electrode as the auxiliary and the reference electrode, respectively.

3. Results and discussion

3.1. Principle of the method

The schematic representation of the competitive assay with p-AP redox cycling for the detection of Aβ(1–42) and total Aβ is illustrated in Fig. 1. The conjugates of Aβ(22–42)–biotin–SA–ALP (Fig. 1B) and Aβ(1–16)-biotin–SA–ALP (Fig. 1C) are captured by pre-immobilized mAb via the antigen-antibody (mAb-Aβ) interaction. After the addition of the p-APP substrate, the enzymatic reaction from p-APP to p-AP by SA-ALP proceeds (Fig. 1D). Note that p-AP is not stable in air and is light-sensitive (Walter et al., 2011). In this sensing system, the auto-oxidation of p-AP would be prevented by reductants. Importantly, in the presence of reductants, p-AP could be cycled after its electro-oxidization on the electrode, enabling an increase in the anodic current. Herein, we chose TCEP as the reductant because we found that TCEP showed low background and fast chemical reaction with QI on MPA-covered gold electrode (see Fig. S1 in Supplementary material). The addition of Aβ into the solution containing conjugates will induce a decrease in the anchored conjugates due to the competitive binding between native Aβ and biotinylated Aβ peptides to mAb. Because Aβ(1–42) mAb can selectively bind to the C-terminus of Aβ(1–42) and Aβ(1–16) mAb is capable of binding

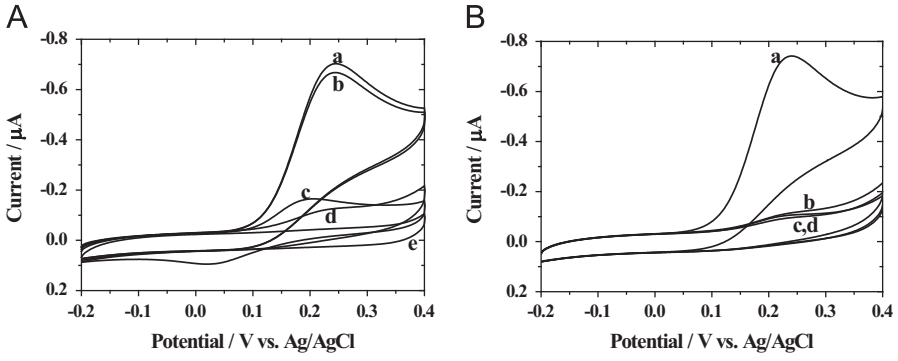

Fig. 2. (A) CVs acquired at Aβ(1–42) mAb-covered electrode after attachment of Aβ(22–42)-biotin-SA-ALP in the absence (curves a and c) and presence (curve d) of Aβ(1–42) and follow-up incubation with p-APP in the absence (curve c) and presence (curves a and d) of TCEP. The control CVs acquired with Aβ(1–40) instead of Aβ(1–42) of curve d or with SA-ALP instead of Aβ(22–42)-biotin-SA-ALP of curve a were shown in curves b and e, respectively. (B) CVs acquired at Aβ(1–16) mAb-covered electrode after attachment of Aβ(1–16)-biotin-SA-ALP in the absence (curve a) and presence of Aβ(1–42) (curve b), Aβ(1–40) (curve c) or Aβ(1–16) (curve d) and follow-up incubation with p-APP in the presence of TCEP. The concentrations of Aβ(1–42), Aβ(1–40) and Aβ(1–16) were 2 nM, and those of biotinylated peptides were 5 nM. The scan rate was 20 mV/s.

to the common N-terminus of Aβ (Wang et al., 2012; Xia et al., 2010), we suggested that the method can be used for the simultaneous detection of Aβ(1–42) and total Aβ in samples.

3.2. Feasibility for Aβ detection

Curves a and c in Fig. 2A show the voltammetric responses acquired at Aβ(1–42) mAb-covered electrode with the capture of Aβ(22–42)-biotin-SA-ALP conjugate and the follow-up incubation with p-APP. Without TCEP, the response can only be from the electro-oxidization of the enzymatically generated p-AP (curve c). Upon the addition of TCEP, the anodic current was greatly enhanced (curve a), showing signal amplification from the redox cycling. In this process, TCEP prevented the auto-oxidation of p-AP in air and regenerated p-AP from its electro-oxidization production QI. For the experiment wherein Aβ(22–42)-biotin was not introduced to the SA-ALP solution, no peak was observed (curve e). The result indicated that conjugation of Aβ(22–42)-biotin allowed SA-ALP to adsorb onto the mAb-modified electrode. Moreover, we found that the anodic current increased with the increase of Aβ (22–42)-biotin concentration and began to level off beyond 5 nM (Fig. S2), indicating that conjugation of Aβ(22–42)-biotin allows SA-ALP to adsorb onto the mAb-modified electrode. In the presence of Aβ(1–42), the competitive binding between Aβ(1–42) and Aβ(22–42)-biotin-SA-ALP to the mAb resulted in a much attenuated peak current (curve d). The selectivity of the method for Aβ (1–42) detection was also assessed with Aβ(1–40) instead of Aβ(1–42). The absence of an apparent difference between curve a and b indicates that the binding of Aβ(1–42) to the anchored mAb is greatly dependent on the C-terminus of Aβ.

For the detection of total Aβ, the same procedure with Aβ(1–42) detection was implemented on electrodes covered with Aβ(1–16) mAb. As shown in Fig. 2B, a catalytic oxidation peak was observed after the capture of Aβ(1–16)-biotin-SA-ALP conjugate and the incubation with p-APP in the presence of TCEP. However, the currents almost dropped to the background level after the addition of Aβ(1–40), Aβ(1–42) or Aβ(1–16). The results are understandable since the Aβ(1–16) mAb can bind to the common N-terminus of Aβ(1–42), Aβ(1–40) and Aβ(1–16) (Wang et al., 2012; Xia et al., 2010). Note that all the native Aβ species in human cerebrospinal fluid (CSF) contain the sequence of Glu–Phe–Arg–His–Asp–Ser (amino acids 3–8 of Aβ) (Golde et al., 2000); thus, the detection of the total Aβ with the proposed method is possible.

3.3. Sensitivity to Aβ

Amperometry is a sensitive and classical electrochemical technique that has been widely applied to detect DNA and proteins. Therefore, amperometry was performed to examine the detection sensitivity of the method. The Aβ samples were prepared by mixing Aβ(1–40), Aβ(1–42) and Aβ(1–16) in a ratio of 6:1:3. The Aβ(1–40)/Aβ(1–42) ratio is close to the real ratio in human CSF (Vestergaard et al., 2005). As seen in Fig. 3, the current decreased with increasing total Aβ concentration ranging from 0.5 to 50 nM at Aβ(1–42) mAb-covered electrode (Fig. 3A) and from 0.05 to 5 nM at Aβ(1–16) mAb-covered electrode (Fig. 3B). The insets in panels A and B show the change in the current values with the concentrations of Aβ(1–42) and total Aβ, respectively. The regression equation of the linear portion can be expressed as i (μA)= 0.394–0.327 [Aβ(1–42)] (nM) (R^2=0.99) at Aβ(1–42) mAb-covered electrode (Fig. 3A) and i (μA)=0.405–0.351 [total Aβ] (nM) at Aβ (1–16) mAb-covered electrode (R^2=0.99) (Fig. 3B). The detection limit of the method, estimated from 3σ of the baseline signals (n=11), was 5 pM for Aβ(1–42) at Aβ(1–42) mAb-covered electrode or for total Aβ at Aβ(1–16) mAb-covered electrode, which is compared to or lower than that achievable by capillary electrophoresis (0.5 μM), SPR (3.5 pM), resonance light scattering (0.8 ng/mL) and gold nanoparticle-based dot-blot immunoassay (50 pg/mL) (Picou et al., 2010; Wang et al., 2011; Wang et al., 2012; Xia et al., 2010). The lower detection limit is attributed to the high turnover frequency of alkaline phosphatase and the dual-amplification of enzymatic reaction plus p-AP redox cycling reaction. Moreover, our method obviates the need of expensive biotin- or enzyme-linked antibody and functionalized nanoparticles for recognition and signal amplification, reducing the operation complexity and assay cost. The basal levels of Aβ(1–42) and total Aβ in human CSF are at nanomolar scale (Ammar et al., 2013; Golde et al., 2000; Xia et al., 2010). Our results demonstrated that electrochemistry holds great potential for the simultaneous detection of Aβ(1–42) and total Aβ in a biological matrix. Moreover, to demonstrate the viability of the sensors for analysis of real samples, we carried out the experiment in aCSF. The results indicated that CSF environment has no interference in the detection (Fig. S3). In addition, we found that the relative standard deviations (RSDs) are below 12% at three different electrodes in parallel, indicating that multiple electrodes can be prepared concurrently for the assay of many different samples.

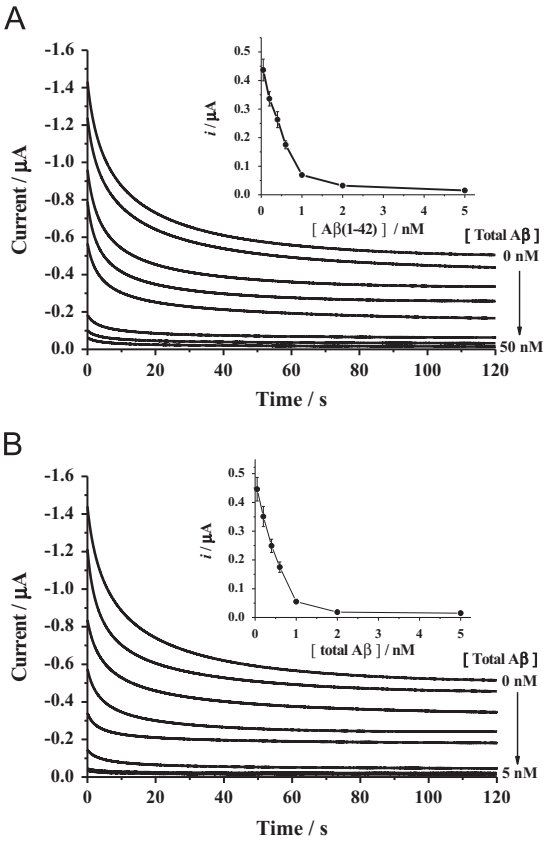

Fig. 3. Amperometric responses at Aβ(1–42) mAb-covered electrode with increasing total Aβ concentrations (0, 0.5, 1, 2, 5, 10, 20 and 50 nM) (A) and at Aβ(1–16) mAb-covered electrode with increasing total Aβ concentrations (0, 0.05, 0.1, 0.2, 0.5, 1, 2 and 5 nM) (B). The concentration ratio of Aβ(1–40)/Aβ(1–42)/Aβ(1–16) is 6:1:3. The current-time curves were obtained at the potential of 0.25 V. The insets show the dependence of the current on the concentration of Aβ(1–42) and total Aβ. The absolute errors were deduced from three replicate measurements and are shown as the error bars.

4. Conclusion

The detection of Aβ(1–42) and total Aβ has important clinical implication. In this work, we first reported an electrochemical method for the simultaneous detection of Aβ(1–42) and total Aβ in

samples using p-AP redox cycling by TCEP. The conjugates of Aβ (22–42)-biotin-SA-ALP and Aβ(1–16)-biotin-SA-ALP captured by the mAb-modified electrodes catalyzed the production of electrochemically active p-AP from the p-APP substrate. Owing to the competitive binding between native Aβ and biotinylated Aβ peptides to the specific mAb on the electrodes, the amount of anchored SA-ALP decreased with the increase of native Aβ concentration, resulting in the attenuation of the oxidation current of p-AP. A detection limit of 5 pM was achieved. The technical simplicity, high sensitivity and selectivity of the method facilitated the measurements of the content of Aβ spiked into aCSF. We believe that our work would be valuable for the early diagnosis of AD and lead to many applications in the design of sensitive electrochemical immunosensors.

Acknowledgments

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.07.047.

References

Akanda, M.R., Choe, Y.-L., Yang, H., 2012. Analytical Chemistry 84, 1049–1055.
Ammar, M., Smadja, C., Giang Thi Phuong, L., Azzouz, M., Vigneron, J., Etcheberry, A., Taverna, M., Dufour-Gergam, E., 2013. Biosensors and Bioelectronics 40, 329–335.
Brookmeyer, R., Johnson, E., Ziegler-Graham, K., Arrighi, M.H., 2007. Alzheimers and Dementia 3, 186–191.
Chen, X., Cheng, X., Gooding, J.J., 2012. Analytical Chemistry 84, 8557–8563.
Das, J., Aziz, M.A., Yang, H., 2006. Journal of the American Chemical Society 128, 16022–16023.
Das, J., Jo, K., Lee, J.W., Yang, H., 2007. Analytical Chemistry 79, 2790–2796.
Doong, R.-A., Lee, P.-S., Anitha, K., 2010. Biosensors and Bioelectronics 25, 2464–2469.
Geng, J., Li, M., Ren, J., Wang, E., Qu, X., 2011. Angewandte Chemie International Edition 50, 4184–4188.
Golde, T.E., Eckman, C.B., Younkin, S.G., 2000. Biochimica et Biophysica Acta 1502, 172–187.
Haes, A.J., Chang, L., Klein, W.L., Van Duyne, R.P., 2005. Journal of the American Chemical Society 127, 2264–2271.
Hardy, J., Selkoe, D.J., 2002. Science 297, 353–356.
Hegnerová, K., Bocková, M., Vaisocherová, H., Krištofiková, Z., Říčný, J., Rípová, D., Homola, J., 2009. Sensors and Actuators B 139, 69–73.
Jiang, B., Wang, M., Chen, Y., Xie, J., Xiang, Y., 2012. Biosensors and Bioelectronics 32, 305–308.
Kang, D.-Y., Lee, J.-H., Oh, B.-K., Choi, J.-W., 2009. Biosensors and Bioelectronics 24, 1431–1436.
Kim, E., Kim, K., Yang, H., Kim, Y.T., Kwak, J., 2003. Analytical Chemistry 75, 5665–5672.
Kwon, S.J., Kim, E., Yang, H., Kwak, J., 2006. The Analyst 131, 402–406.
Liu, L., Deng, D., Xing, Y., Li, S., Yuan, B., Chen, J., Xia, N., 2013a. Electrochimica Acta 89, 616–622.
Liu, L., Du, J., Li, S., Yuan, B., Han, H., Jing, M., Xia, N., 2013b. Biosensors and Bioelectronics 41, 730–735.
Liu, L., Jiang, D., McDonald, A., Hao, Y., Millhauser, G.L., Zhou, F., 2011. Journal of the American Chemical Society 133, 12229–12237.
Moreno-Guzmán, M., Ojeda, I., Villalonga, R., González-Cortés, A., Yánez-Sedeño, P., Pingarrón, J.M., 2012. Biosensors and Bioelectronics 35, 82–86.
Mustafa, M.K., Nabok, A., Parkinson, D., Tothill, I.E., Salam, F., Tsargorodskaya, A., 2010. Biosensors and Bioelectronics 26, 1332–1336.
Ogi, H., Fukushima, M., Uesugi, K., Yagi, H., Goto, Y., Hirao, M., 2013. Biosensors and Bioelectronics 40, 200–205.
Ojedaa, I., López-Monteroa, J., Moreno-Guzmána, M., Janegitzb, B.C., González-Cortésa, A., Yáñez-Sedeñoa, P., Pingarróna, J.M., 2012. Analytica Chimica Acta 743, 117–124.
Patolsky, F., Lichtenstein, A., Willner, I., 2000. Angewandte Chemie Internation Edition 39, 940–943.
Picou, R., Moses, J.P., Wellman, A.D., Kheterpal, I., Douglass Gilman, S., 2010. The Analyst 135, 1631–1635.
Rolinski, O.J., Amaro, M., Birch, D.J.S., 2010. Biosensors and Bioelectronics 25, 2249–2252.
Southwick, P.C., Yamagata, S.K., Echols, J., Higson, C.L., Neynaber, G.J., Parson, S.A., Munroe, W.A., R.E., 1996. Journal of Neurochemistry 66, 259–265.
Stravalaci, M., Beeg, M., Salmona, M., Gobbi, M., 2011. Biosensors and Bioelectronics 26, 2772–2775.
Venkatanarayanan, A., Keyes, T.E., Forster, R.J., 2013. Analytical Chemistry 85, 2216–2222.
Vestergaard, M., Kerman, K., Saito, M., Nagatani, N., Takamura, Y., Tamiya, E., 2005. Journal of the American Chemical Society 127, 11892–11893.
Walter, A., Wu, J., Flechsig, G.-U., Haake, D.A., Wang, J., 2011. Analytica Chimica Acta 689, 29–33.
Wang, C., Liu, D., Wang, Z., 2011. Chemical Communications 47, 9339–9341.
Wang, C., Liu, D., Wang, Z., 2012. Chemical Communications 48, 8392–8394.
Xia, N., Deng, D., Zhang, L., Yuan, B., Jing, M., Du, J., Liu, L., 2013a. Biosensors and Bioelectronics 43, 155–159.
Xia, N., Liu, L., Harrington, M.G., Wang, J., Zhou, F., 2010. Analytical Chemistry 82, 10151–10157.
Xia, N., Ma, F., Zhao, F., He, Q., Du, J., Li, S., Chen, J., Liu, L., 2013b. Electrochimica Acta, http://dx.doi.org/10.1016/j.electacta.2013.07.118.
Yang, H., 2012. Current Opinion in Chemical Biology 16, 422–428.
Yasukawa, T., Yoshimoto, Y., Goto, T., Mizutani, F., 2012. Biosensors and Bioelectronics 37, 19–23.
Zhuang, X.M., Wang, D.L., Lin, Y.Q., Yang, L.F., Yu, P., Jiang, W., Mao, L.Q., 2012. Analytical Chemistry 84, 1900–1906.