Biosensors Hot Paper

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A Method for Evaluating the Level of Soluble β-Amyloid$_{(1–40/1–42)}$ in Alzheimer's Disease Based on the Binding of Gelsolin to β-Amyloid Peptides**

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Abstract: *In the present work, a new electrochemical strategy for the sensitive and specific detection of soluble β-amyloid Aβ$_{(1–40/1–42)}$ peptides in a rat model of Alzheimer's disease (AD) is described. In contrast to previous antibody-based methods, β-amyloid$_{(1–40/1–42)}$ was quantified based on its binding to gelsolin, a secretory protein present in the cerebrospinal fluid (CSF) and plasma. The level of soluble β-amyloid peptides in the CSF and various brain regions were found with this method to be lower in rats with AD than in normal rats.*

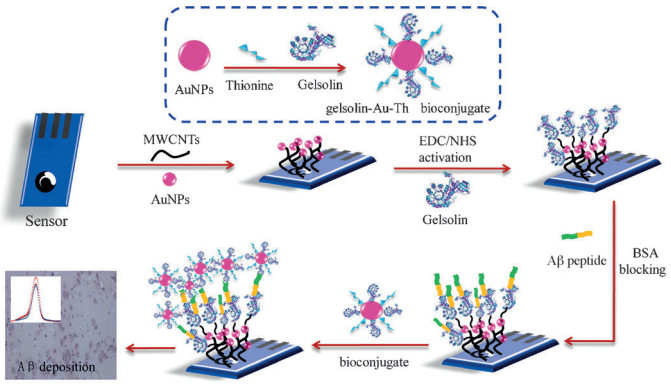
With the aging of the world population, Alzheimer's disease (AD) has become an increasing and serious public health problem in the industrialized world. AD is accompanied by a gradual loss of cognitive function and synaptic integrity, selective neuronal death, and the abnormal formation of neurotic and core plaques in the cerebral cortex.[1]

The aggregation of the amyloid β-peptide (Aβ) into oligomers or fibrils is now implicated as a key factor associated with the progression of AD.[2] Aβ is a 4 kDa peptide cleaved from the amyloid precursor protein that is present in the brain and cerebrospinal fluid (CSF) and it has distinct N and C terminals.[3] In its native form, Aβ is unfolded but it aggregates into a β-sheet structure of ordered fibrils under various conditions.[4] The amount of extracellular soluble Aβ in the brain has been considered a key predictor of cognitive impairment in AD.[5] Two major C-terminal variants of Aβ, Aβ$_{(1–40)}$ and Aβ$_{(1–42)}$, have been identified in amyloid deposits from the brains of patients with AD.[6] Both the ratio of Aβ$_{(1–40)}$ to Aβ$_{(1–42)}$ and the total amount of the two variants have been suggested as criteria for the early diagnosis of AD.[7]

Most of the current methods for detecting Aβ still rely on postmortem identification, which is inevitably influenced by the age of the cohort sampled and how the disease is defined.[8] Although a highly sensitive, selective, and reliable enzyme-linked immunosorbent assay (ELISA) can be used to detect Aβ from body fluids, this method has inherent shortcomings in that it is labor-intensive and requires relatively costly enzyme-linked antibodies for Aβ recognition, as well as carcinogenic substrates for chemiluminescence detection.[7d,9]

Gelsolin, a secretory protein, was reported to bind Aβ in a concentration-dependent manner in 1990, and this result was confirmed by performing the gelsolin overlay assay on Aβ fixed on the nitrocellulose membrane. The specificity of gelsolin for Aβ was shown to be applicable to both Aβ$_{(1–40)}$ and Aβ$_{(1–42)}$.[10] Moreover, the 94 kDa band observed by Western blotting, which corresponds to Aβ + gelsolin, suggests that gelsolin binds to the Aβ monomer and not to its oligomeric forms. These findings give us a hint that the specific binding of Aβ by gelsolin, somewhat like the interaction between an antibody and antigen, could be exploited in the development of a novel electrochemical assay for the detection of total soluble monomeric Aβ$_{(1–40/1–42)}$. Compared to other strategies, this method is easier to perform and does not require antibodies or other costly reagents for Aβ capture.

The principle of the gelsolin-based electrochemical assay for Aβ detection is illustrated in Scheme 1. The biosensor was constructed by using multiwalled carbon nanotubes (MWCNTs) and gold nanoparticles (AuNPs) as the substrate in order to 1) immobilize gelsolin onto screen-printed carbon electrodes (SPCEs) and 2) facilitate electron transfer on the



Scheme 1. A schematic illustration of the electrochemical detection of Aβ$_{(1–40/1–42)}$ by using a gelsolin-Au-Th bioconjugate as a probe.

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film. The sensors were fabricated by using the "sandwich" method that has been previously reported.[11] First the $A\beta_{(1-40/1-42)}$ species are specifically recognized by the gelsolin tethered to the biosensor. This is followed by the binding of gelsolin-Au-Th bioconjugates, which feature gelsolin and thionine (Th) labels linked to AuNPs. The electrochemical detection of Th reduction was used for the quantitative analysis of Aβ in phosphate-buffered saline (PBS) solution (pH 7.4). The established detection assay was demonstrated to have reasonable sensitivity and selectivity, and it can be reliably transferred to evaluate soluble $A\beta_{(1-40/1-42)}$ levels in both the cerebrospinal fluid (CSF) and brain tissues of normal and AD rats, thus providing a useful tool to study the changes that occur in neurodegenerative diseases. To our knowledge, this is the first report of an electrochemical assay that determines the level of a biomarker associated with AD development based on its interaction with gelsolin.

The fabricated sensor exhibited a reduction peak at −0.15 V, which corresponds to the electrochemical reduction of Th (Figure 1A). In this assay, a film made up of both AuNPs and MWCNTs was used as the substrate, which was designed for electron-transfer acceleration and mass loading of proteins to achieve a high sensitivity. Figure 1B clearly shows that employing MWCNTs and AuNPs together endows a higher response compared to MWCNTs or AuNPs alone. Gelsolin concentration also plays an important role in sensor

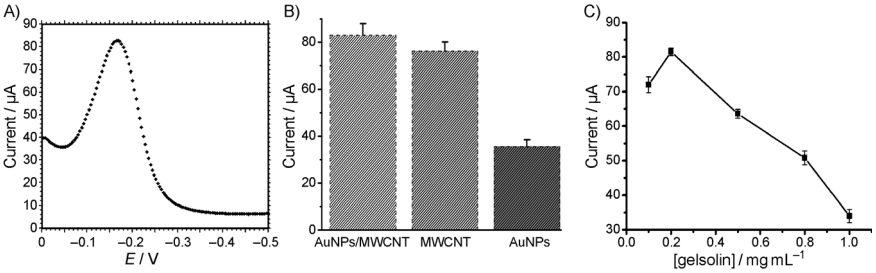

Figure 1. A) Differential pulse voltammetry (DPV) response on the fabricated $A\beta_{(1-40/1-42)}$ sensor in PBS (0.1 M, pH 7.4). B) Comparison of the DPV responses toward 20 nM $A\beta_{(1-40/1-42)}$ when using MWCNTs, AuNPs, and MWCNTs/AuNPs as the substrate. C) The influence of gelsolin concentration (0.1–1.0 mg mL^{-1}) on the DPV responses toward 20 nM Aβ.

fabrication since the binding of Aβ to gelsolin is directly dependent on the concentration of gelsolin. A maximum response was achieved with a gelsolin concentration of 0.2 mg mL^{-1}, above which, the current tended to decrease (Figure 1C). Although more gelsolin would bind more Aβ, mass deposition of poorly conductive proteins would hinder the electron transfer on the sensor.

The binding interaction between gelsolin and $A\beta_{(1-40/1-42)}$ was investigated by using immunoprecipitation and electrochemical techniques (see Figure S5 in the Supporting Information). Figure 2A shows the selectivity of the present detection assay. When there was no Aβ on the sensor, the reduction peak at −0.15 V disappeared owing to the absence of Th and the current decreased markedly. Neither shorter Aβ sequences, such as $A\beta_{(1-11)}$ and $A\beta_{(1-16)}$, nor Aβ oligomers and aggregated forms, including protofibrils and fibrils, interfered with the detection of $A\beta_{(1-40/1-42)}$ since fibrils and protofibrils

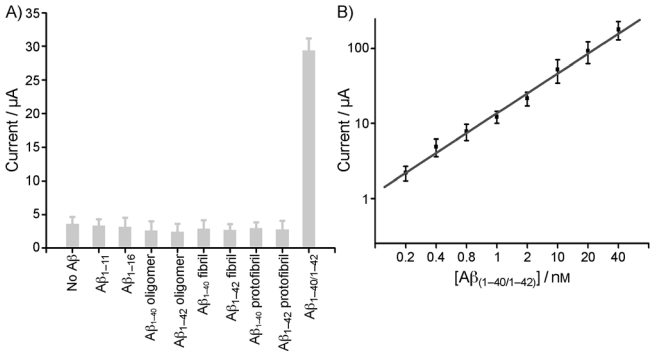

Figure 2. A) The selectivity of the optimized assay for detecting Aβ over potential sources of interference. B) A linear plot of the peak currents at −0.15 V as a function of $A\beta_{(1-40/1-42)}$ concentration.

are insoluble and the specific binding occurred exclusively between soluble Aβ monomers and gelsolin. The interactions of $A\beta_{(1-11)}$ and $A\beta_{(1-16)}$ with gelsolin were considerably weaker than those of longer Aβ variants, as evidenced by protein docking and molecular dynamics simulation results (see Figure S6 in the Supporting Information). Notably, although Aβ oligomers are also soluble, their interaction with gelsolin was much weaker than that of the monomers because the conformational change of Aβ from α-helix to β-sheet that occurs during the formation of oligomers is not beneficial for binding.[12] Therefore, the response to sensors modified with oligomeric Aβ was also remarkably low. Beside Aβ variants, interference from other proteins such as actin, tyrosine kinase 2β, and voltage-dependent anion channel 1, which have been reported to interact with gelsolin, were also investigated.[13] Herein, we present actin as an example. As can be observed from Figure 3A, the reduction peaks for Aβ and actin located to −150 and −300 mV, respectively, with a 150 mV potential gap between them. This gap is considered to be large enough to separate actin from Aβ determination. In addition to a peak potential that is more positive than that for actin, the peak shape for Aβ also seemed to be more sharp and regular than that obtained for actin. Moreover, the similarity of the DPV response obtained for the hippocampus homogenate to that of $A\beta_{(1-40/1-42)}$ solution at −0.15 V and the stepwise increase in current at −0.15 V with the addition of $A\beta_{(1-40/1-42)}$ solutions to the tissue (Figure 3B) indicate that it is Aβ that contributes to the production of current signals at −0.15 V and not actin or other contributors since the peak ascribed to actin didn't even appear under this condition. This result might be caused by the low amount of dissociated actin in the homogenate compared with Aβ. In light of this, actin would not interfere with Aβ determination at the potential of −0.15 V even when they coexist in the homogenate. Similar results were obtained for tyrosine kinase 2β and voltage-dependent anion channel 1 proteins, with 210 and 130 mV potential gaps between them and Aβ, respectively (see

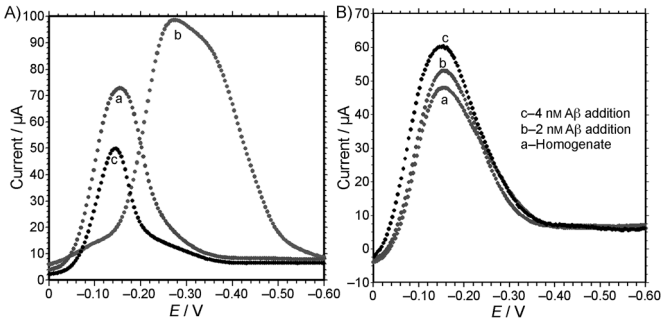

Figure 3. A) DPV responses to 18 nM $A\beta_{(1-40/1-42)}$ (a), 10 nM actin standard solution (b), and hippocampus homogenate from AD rats (c). B) DPV responses to hippocampus homogenate with the addition of different concentrations of $A\beta_{(1-40/1-42)}$ standard solution.

Table 1: Evaluation of the variation in $A\beta_{(1-40/1-42)}$ levels in normal, control, and AD rat brains.

	Aβ content [nM][a]			
	CSF	hippocampus	prefrontal cortex	striatum
normal[c]	18.28 ± 2.26	15.51 ± 1.73	8.85 ± 0.84	5.42 ± 0.50
control[d]	17.95 ± 1.43	15.05 ± 2.54	8.79 ± 1.98	5.38 ± 1.47
AD	4.42 ± 0.63	7.70 ± 1.05	5.76 ± 0.78	4.29 ± 0.44
decrease [%][b]	75.8	50.4	34.9	20.8

[a] $n = 10$, mean ± SD. [b] Percentage difference in Aβ content between normal and AD brains. [c] Untreated healthy rats. [d] Rats administrated with D-gal daily for six weeks and then given bilateral infusions of 1 μL saline.

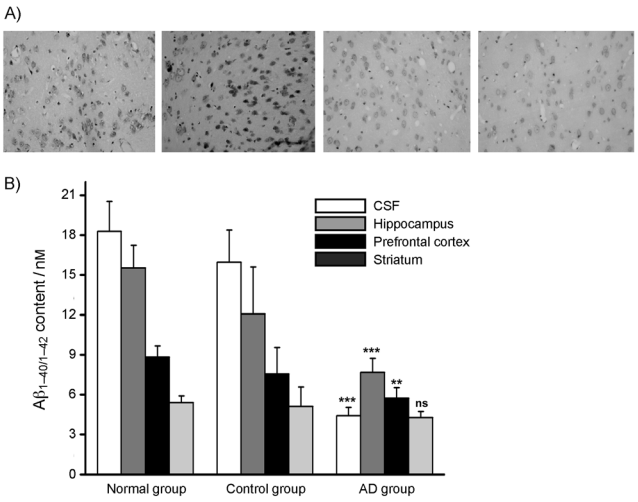

Figure 4. A) $A\beta_{(1-40/1-42)}$ deposition in CSF and fixed brain tissue from AD rats. From left to right: CSF, hippocampus, prefrontal cortex, and striatum. B) Percentage reduction of soluble $A\beta_{(1-40/1-42)}$ in the CSF, hippocampus, prefrontal cortex, and striatum in AD rats compared to normal rats. $^{**}P < 0.01$, $^{***}P < 0.001$ vs. normal rats, ns = not significant.

Figure S7 in the Supporting Information). These results signify the high selectivity of the present method for $A\beta_{(1-40/1-42)}$ relative to other isoforms of Aβ, including soluble and insoluble forms, and other gelsolin-binding proteins.

Other parameters such as linear range, detection limit, and reproducibility were also evaluated. The logarithm of the peak current ($\log i_p$) from DPV showed a linear relationship with $A\beta_{(1-40/1-42)}$ in the concentration range of 0.2–40 nM. The linear relationship thus spans three orders of magnitude before reaching a plateau at higher concentrations (Figure 2B). The limit of detection was approximately 50 pM (three times the standard deviation of the blank, 3σ) with a relative standard deviation of 1.30% ($n = 6$), which is similar or superior to that reported for other assays.[14]

As demonstrated above, the electrochemical method for detecting $A\beta_{(1-40/1-42)}$ at nanomolar levels with high selectivity could be used to assess $A\beta_{(1-40/1-42)}$ levels in the brains of normal and AD rats. From the relationship between current and the amount of $A\beta_{(1-40/1-42)}$ present, the concentration of $A\beta_{(1-40/1-42)}$ in the CSF of normal rats was estimated to be 18 nM. It was previously reported that the relative abundance of $A\beta_{(1-40)}$ and $A\beta_{(1-42)}$ in the CSF is approximately 60–70% and 5–15%, respectively, with $A\beta_{(1-42)}$ concentrations in the range of 0.3–5 nM in CSF.[14c,15,16] The total amount of $A\beta_{(1-40/1-42)}$ in the CSF should thus be around 1.5–25 nM. The value obtained here was within this range. In AD rats, the $A\beta_{(1-42)}$ concentration was 4.4 nM, which is 76% lower than in normal animals (Table 1). It has been reported that Aβ peptides, especially $A\beta_{(1-42)}$, have a propensity to form oligomers and higher-order protofibrils and fibrils. Soluble Aβ monomer levels in the CSF negatively correlate with the concentration of insoluble β-amyloid aggregates in brain tissue and therefore decrease with AD progression.[17]

To further demonstrate the reliability of our detection assay, the gelsolin-based detection assay was performed with tissue from the hippocampus, prefrontal cortex, and striatum (Table 1 and Figure 4B). The $A\beta_{(1-40/1-42)}$ levels in these areas were lower in AD compared to normal rats, a result consistent with previous observations that $A\beta_{(1-40/1-42)}$ aggregation in AD occurs not only in the CSF but also in the brain tissues.[17b,18] The reduction in the levels of soluble $A\beta_{(1-40/1-42)}$ monomers was greatest in CSF, followed by the hippocampus, cortex, and striatum. Meanwhile, a histological examination of tissue sections stained with Congo red also revealed distinguishable degrees of Aβ aggregation in the different brain sections of AD rats (Figure 4A). As reported, one pathological feature of AD is the extracellular deposition of Aβ in the form of neurotic plaques that cause lesions in the prefrontal cortex and hippocampus.[19] This fact forms the basis of the AD–Aβ association theory. The aggregates are initially formed in the prefrontal cortex and hippocampus,[20] which are the brain areas responsible for memory and cognitive function. The cognitive decline associated with AD might thus directly result from Aβ accumulation.

In summary, a novel gelsolin-based electrochemical probe that exploits the amplifying capabilities of AuNPs and can specifically bind $A\beta_{(1-40/1-42)}$ was designed and it was used to quantify the changes in the level of these peptides in AD-affected rat brains. The simplicity, sensitivity, and selectivity of this assay make it potentially suitable for the clinical diagnosis of human patients. However, given that $A\beta_{(1-42)}$ aggregates more readily and is more toxic than $A\beta_{(1-40)}$,

developing a means of discriminating between these variants will be an important goal for future studies.

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