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Peptide-based electrochemical biosensor for amyloid β 1–42 soluble oligomer assay

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ABSTRACT

Based on oligopeptide, a novel strategy to fabricate electrochemical biosensor is proposed in this work by fine-tuning the scan pulse frequency of square wave voltammetry (SWV) to synchronize with the surface electron transfer (ET) of the oligopeptide modified on an electrode surface. By using this strategy, the surface ET dynamics of our peptide-based biosensor can show significant difference in the presence and absence of a detection target, thus the proposed strategy has been employed for the assay of amyloid β 1–42 (Aβ 1–42) soluble oligomer, which is among the most neurotoxic species of Aβ peptide. Experimental results reveal that our sensor might be an appropriate candidate for quantitative assay of Aβ 1–42 soluble oligomer. Moreover, the strategy proposed in this work may be extended for the fabrication of more peptide-based biosensors in the future.

1. Introduction

Design and development of functional oligopeptides have gradually progressed in the past few years [1]. These oligopeptides can recognize molecular partners of biological significance such as key components in signal transduction pathways, surface antigen of tumor cells and other biomarkers even in the absence of protein scaffold [2–8]. Therefore, these oligopeptides have wide applications in various fields as the lead compounds for drug discovery, as agents intervening in pathological process, as building blocks in tissue engineering, etc. [9–12]. However, oligopeptides have not been well integrated into biosensor design, although their properties may give this kind of molecule the intrinsic advantage as the recognizing component of a biosensor [13,14].

Recently, works utilizing peptide strand as the probe of interface sensing system have been reported [15–17]. Since electrochemistry is a cost-effective method with high sensitivity, reliability and convenience in constructing biosensors, more work on electrochemical peptide-based biosensors should be conducted. Therefore, in this work we would present a novel method for fabrication of electrochemical biosensor by making use of functional oligopeptides.

On the other hand, it has been known that any change in electron transfer through self-assembly monolayer (SAM) on an electrode surface can be reflected through the change of sensor response [18–20]. Moreover, since a signal-on response can be achieved by tuning the scan pulse frequency of square wave voltammetry (SWV), involving no more complexity in sensor design, DNA-based sensors have thus been proposed by fine-tuning SWV frequency to synchronize with surface ET dynamics [21,22]. Oligopeptides and DNA strands are somewhat analogous; however they are not all the same in electrostatic interaction, backbone elasticity, secondary structure and ET, hence the SAMs of them may display different dielectric and ET qualities. Therefore, we have proposed a novel peptide-based sensing strategy in this work by making use of the analyte-binding induced change of surface ET, as well as the fine-tuned SWV frequency. This strategy has also been demonstrated by fabricating a signal-on biosensor for the quantitative determination of amyloid β 1–42 (Aβ 1–42) soluble oligomer, which is regarded as the major pathogenic factor of neuronal degradation in Alzheimer's disease (AD) [23].

2. Experimental

2.1. Materials and chemicals

The peptide probe (11-mercaptoundecanoic acid MUA-RGTWEGKWK-Ferrocene (Fc), I10 peptide [24] as the probe of Aβ 1–42 soluble oligomer (Fig. S1, supporting information (SI),

Abbreviations: ET, electron transfer; Aβ 1–42, amyloid β 1–42; SAM, self-assembly monolayer; MUA, 11-mercaptoundecanoic acid; Fc, Ferrocene; HFIP, 1,1,1,3,3,3-hexafluoro-2-propanol; TCEP, Tris(2-carboxyethyl) phosphine hydrochloride; MNH, 9-mercapto-1-nonanol; DMSO, dimethyl sulfoxide; AD, Alzheimer's disease.

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Section I), and MUA-RWKNFIAVSAANRFKKISK-Fc, M13 peptide [25] as the control peptide sequence) were custom-synthesized by Chinapeptide Co., Ltd. Aβ 1–42 (>98%), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), tris(2-carboxyethyl) phosphine hydrochloride (TCEP) and 9-mercapto-1-nonanol (MNH) were obtained from Sigma–Aldrich. Other reagents were all of analytical-grade. The stock solution of peptide probe was prepared with dimethyl sulfoxide (DMSO) and diluted to 5 μM with 10 mM phosphate buffer solution (pH 7.4). All solutions were prepared with double-distilled water, which was purified with a Milli-Q purification system (Branstead, USA) to a specific resistance of 18 MΩ cm.

2.2. Preparation of Aβ 1–42 soluble oligomer

Aβ 1–42 soluble oligomer was prepared according to literature [26]. Briefly, Aβ 1–42 lyophilized powder was firstly dissolved in HFIP at a concentration of 1 mg/mL to monomerize pre-existing aggregates. After that, HFIP was evaporated under a gentle stream of high purity nitrogen, leaving a film which was later re-dissolved in DMSO to 2.2 mM. The resuspension was further monomerized by sonication for 1 min, followed by being diluted to 440 μM (the oligomerization process would be hardly initiated below this concentration [27]) with 10 mM phosphate buffered saline (PBS) (pH 7.4), and subsequently being incubated at 4 °C for 24 h. The above prepared solution of soluble oligomer was transparent without evident aggregation. Gel filtration experiment was performed to analyze their fractions, and the results are shown in Fig. S2, SI, Section I. In monomer control, the 440 μM suspension was firstly centrifuged at 12,000 × *g*, and then diluted immediately to 12 nM. In aggregated target control, the 440 μM suspension was acidified to pH 2.0 by HCl and then left at room temperature for one week to form a fibrillar preparation. In the serum sample, the stock solution was diluted by fetal bovine serum to desired concentrations.

2.3. Gold electrode treatment and modification

The substrate gold disk electrode (3 mm diameter) was firstly cleaned with piranha solution (70% concentrated sulfuric acid, 30% H_2O_2) for 5 min followed by rinsing with double-distilled water (*Caution: piranha solution reacts violently with organic solvents and should be handled with great care!*). Then the electrode was polished with 1 μm, 0.3 μm, and 0.05 μm alumina slurry in sequence. Residual alumina powder was thoroughly removed by ultrasonicating in both ethanol and water for 5 min, respectively. Finally, the electrode was soaked in nitric acid (50%) for 30 min, and electrochemically cleaned with 0.5 M H_2SO_4 to remove any remaining impurities.

After being dried with nitrogen, the pre-treated electrode was firstly incubated in the assembly solution (10 mM PBS, pH 7.4, containing 2.5 μM peptide probe and 5 mM TCEP) for 16 h at 4 °C. TCEP was used to prevent terminal mercapto groups of the peptide probes from forming disulphide. Then, the electrode was immersed into 100 μL MNH solution (1 mM in 10 mM PBS, pH 7.4) for 3 h at room temperature. Finally, the modified electrode was thoroughly rinsed to remove MNH adsorbed on the electrode surface followed by being dried under mild nitrogen stream.

For the measurement of Aβ 1–42 soluble oligomer, the above prepared modified electrode was firstly treated with different concentrations of Aβ 1–42 soluble oligomer in 10 mM PBS (pH 7.4) for 20 h at room temperature. After that, the electrode was thoroughly rinsed with double-distilled water. Finally, the electrode was dipped in 5% Tween-20 for 30 min to exclude unspecific adsorption. For the M13 peptide control, an electrode modified with M13 peptide was subject to the above described treatment.

2.4. Electrochemical measurements

Electrochemical measurements were carried out on a CHI660C Potentiostat (CH Instruments) with a conventional three-electrode cell at room temperature. The three-electrode system consisted of the modified electrode as the working electrode, a saturated calomel electrode (SCE) as the reference electrode, and a platinum wire as the counter electrode. A 1 M sodium perchlorate buffer ($NaClO_4$) was adopted as the electrolyte solution. Cyclic voltammograms (CV) were recorded in the potential scan range from 0.65 to 0.2 V at a scan rate of 4 V/s, and SWV was performed within the potential range from 0 to 1.0 V under modulation amplitude of 25 mV and various scan rates with a step potential of 4 mV. CV scan was also performed in the potential range from 0.7 to 0 V by using 0.1 M KCl containing 5 mM $Fe(CN)_6^{3-/4-}$ as electrolyte solution. All the electrochemical measurements were performed by independent experiments with repetition for at least three times, and the error bars have been shown in figures.

3. Results and discussion

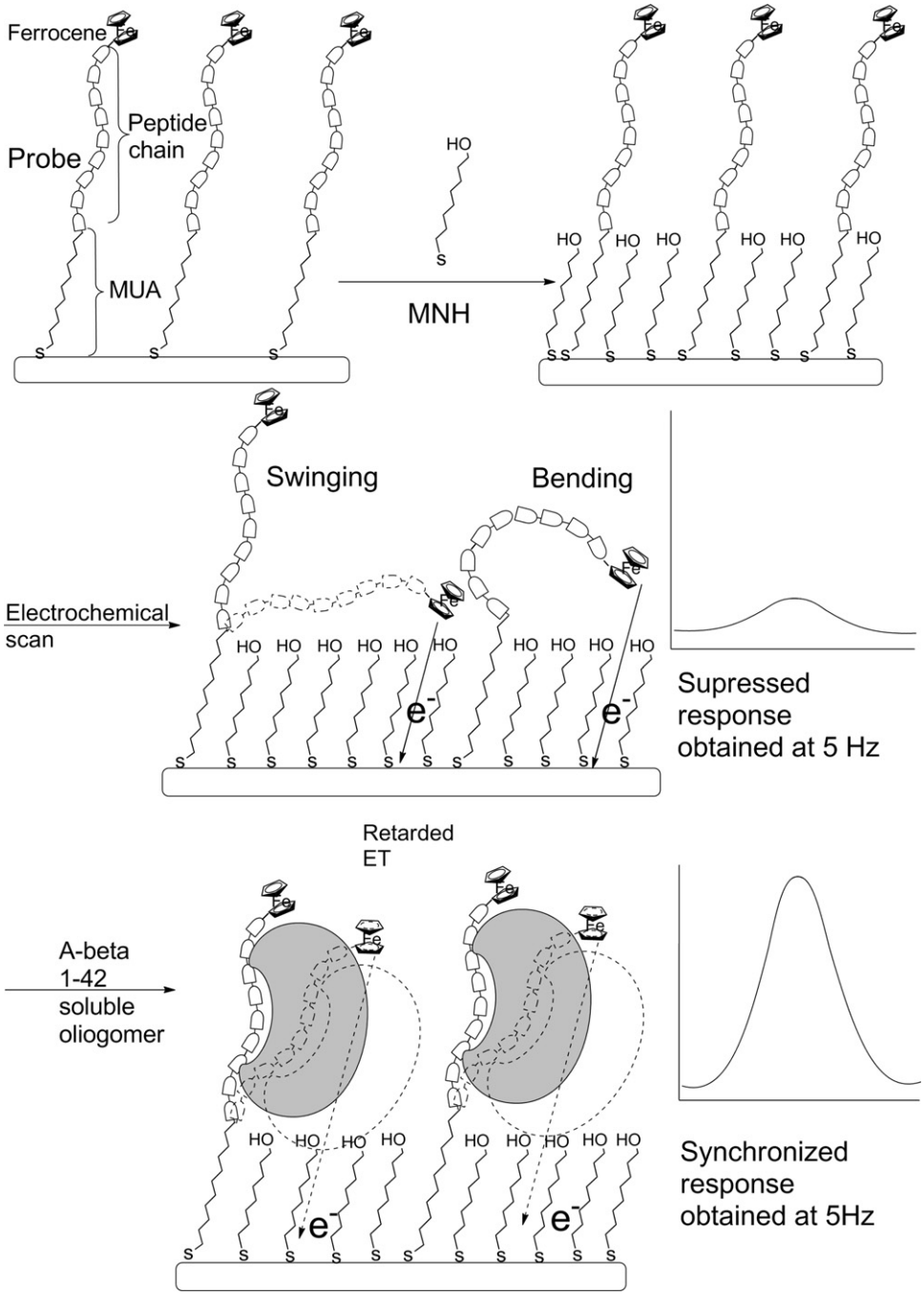
3.1. Electrochemical examination of surface modification

Scheme 1 illustrates the strategy to fabricate the peptide-based electrochemical biosensor, where Aβ 1–42 soluble oligomer is used as the target. The peptide, linked with an Fc at one terminal and an alkyl thiol at the other, and MNH molecules are sequentially assembled on the electrode surface given the fact that thiols have a strong affinity with the substrate gold. On such a prepared SAM, the peptide chains would project out of the surface layer of alkyl chains, bending or curbing freely, since the MUA linker of the peptide probe is slightly longer than the dilution agent MNH. It should be mentioned that the surface density of the peptide probe is an important parameter in the construction of our sensor. Experimental results have shown that the optimal density of the peptide probe loaded on the gold surface is about 3.20×10^{-12} mol cm^{-2} (see SI, Section II, Fig. S3), indicating that the average distance between two probe molecules is big enough for bending, swinging and probe–target interaction without apparent stereo hindrance and electrostatic interference.

3.2. Electrochemical characterization of peptide–target interaction

The linear peptide probe recognizes a special motif on the surface of Aβ 1–42 soluble oligomer and captures the target in a ridge-notch matching style through hydrophobic interactions between amino acid side chains [24]. And the binding of the peptide probe with the targets is thought to cause a significant change of surface ET rate, as is illustrated in Scheme 1. To estimate the influence of the target-binding on the ET rate, SWV measurements have been conducted at various frequencies for target-free and target-stiffened probes, respectively.

Fig. 1 depicts the SWV responses obtained at four typical frequencies. As shown in Fig. 1a, a strong peak around 500 mV can be observed when applying SWV scan to the target-bound sensor at $f = 5$ Hz; while the target-free sensor results in suppressed response under the same condition. Instead, at $f = 20$ Hz, 40 Hz and 80 Hz, a signal-off discrimination of target-free/-bound sensor is achieved (Fig. 1d). These results are analogous to those of DNA-based SWV sensors [21,22] and demonstrate that target binding may induce slowdown of surface ET. The relatively slow ET of the target-bound sensor may synchronize with slow SWV scan, namely, the slow ET of the target-bound sensor takes just as long as the time span of a relatively long SWV pulse to accomplish. Hence the target-bound



Scheme 1. Schematic illustration of the fabrication and electrochemical principle of the peptide-based biosensor.

sensor has an amplified response at low frequencies, but its slow surface ET process becomes more and more desynchronized with gradually faster SWV scans, resulting in suppressed responses at these high frequencies [21,22,28–30].

SWV responses are thus obtained at wider range of frequencies and summarized as an I_p/f–$1/f$ plot in order to confirm our preliminary conclusion that target-binding diminishes surface ET rate. In theory, a specific surface ET process synchronizes with a specific SWV frequency, which is proportionally larger for faster surface ET processes. Except for extremely high SWV frequencies, the normalized current response, I_p/f, reflects the extent of synchronization. If the surface ET process may better synchronize with an SWV frequency, a larger I_p/f value will be obtained at this frequency [22,28–30]. Consequently, altered surface ET rate will lead to different trend on the plot, as observed on our sensor where the opposite trends of curves and their crossover are present (see SI, Section III, Fig. S4). As can be seen, SWV frequencies lower than 20 Hz synchronize with the target-stiffened sensor better than with the target-free sensor, as the former has larger I_p/f value on the plot than the latter, while the situation is reversed for frequencies larger than 20 Hz. This phenomenon is present at different target concentrations and grows relatively weaker for lower target concentration, coherent with the fact that the sensor response is the collective reflection of all probe molecules on the electrode surface. These observations verify us the slow-down of surface ET rate and correlate with the behavior of surface ET through peptide probe mobility. The capture of target will make the probe–target complex much more bulky and cumbersome than the target-free probe,

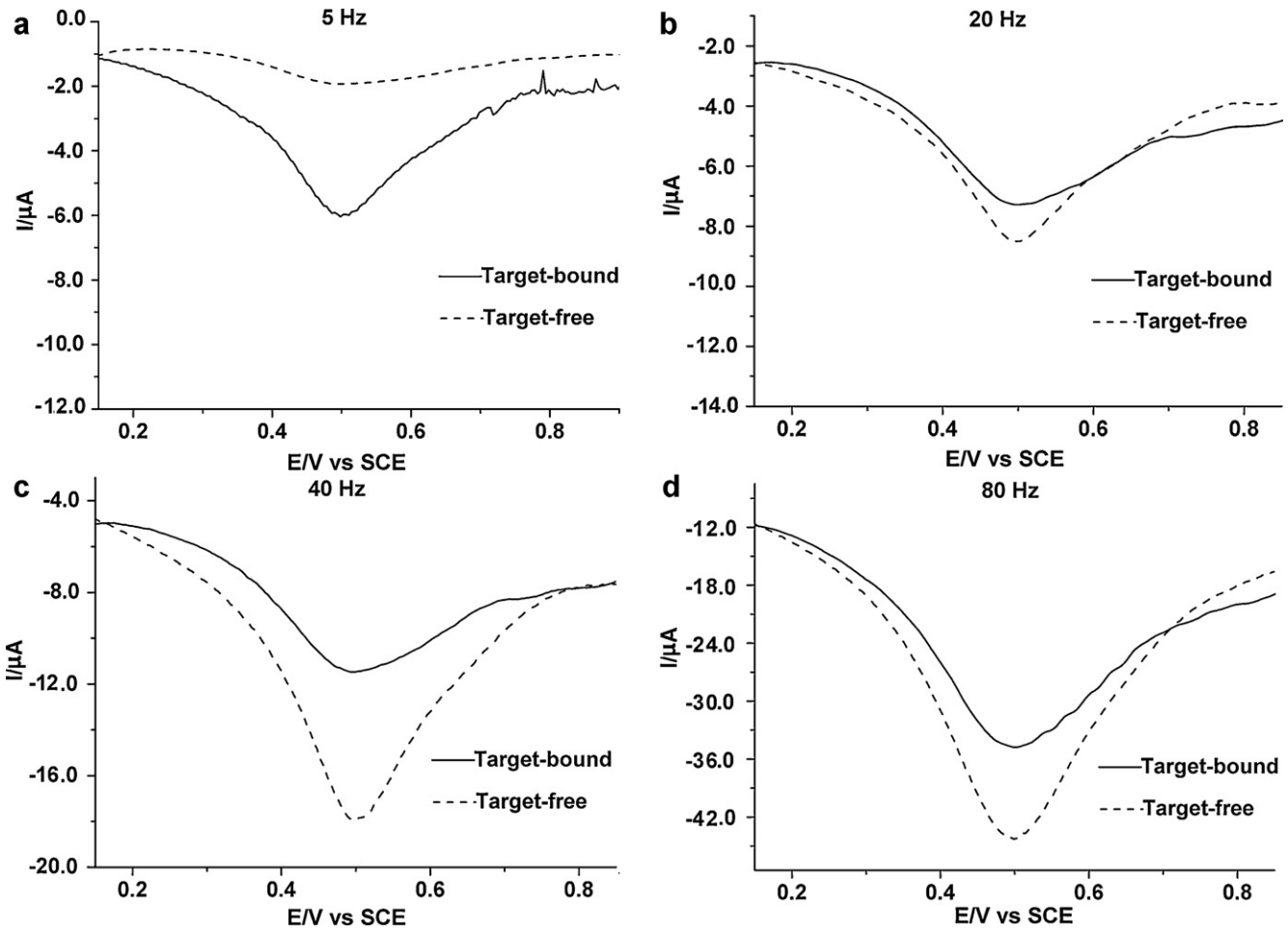


Fig. 1. SWV responses at (a) 5 Hz, (b) 20 Hz, (c) 40 Hz and (d) 80 Hz in 1 M $NaClO_4$ before and after the incubation of the sensor with a 10 mM pH 7.4 PBS solutin containing Aβ 1–42 soluble oligomer. Target concentrtion (equivalent monomer): 12 nM.

resulting in an effective strand length longer than that of the target-free probe, which in turn lengthens the average distance between Fc and the surface layer, and consequently diminishes the rate of surface ET. It is interesting that such behavior is in accord with that of the reported DNA-based SWV sensors, which has been ascribed to the binding-induced changes in DNA strand flexibility [21,22].

3.3. Optimizing SWV frequency for the signal-on assay

The above delineation of surface ET change might have demonstrated clearly the advantage of SWV to distinguish ET processes with different ET rates. Particularly, when the ET rate of a certain ET process alters, a new frequency will be needed to synchronize with it. Thus the response at the original frequency will become suppressed and *vice versa*. This may enable us to achieve signal-on observation at low frequencies, as target-binding will slow down the surface ET as observed above. At low frequencies, the SWV response from the target-free probe is selectively suppressed, while the response from the target-stiffened probe is selectively retained. Specifically, our I_p/f–$1/f$ plots shown in Fig. S4 have revealed that the maximal differentiation is achieved at around 5 Hz, we therefore decide to use SWV response at 5 Hz to demonstrate the analytical performance of our peptide-based electrochemical sensor.

3.4. Detection of Aβ 1–42 soluble oligomer

Recent studies have revealed that the aggregated fibrillar entanglement of amyloid β involves in complicated dynamic equilibrium with various low abundance soluble intermediates [31], among which the soluble oligomer is more and more accepted as the principal cytotoxic agent in Alzheimer's disease [23]. Therefore a biosensor specifically recognizing soluble oligomer may hold more promise in potential clinical applications.

3.4.1. Optimizing condition for target incubation

Works dealt with Aβ 1–42 peptide adopt overnight incubation as the standard condition for Aβ 1–42–ligand interaction [24,26]. We have thus traced the time course of target-binding at 24 nM to make sure that the maximal increment of sensor response at 5 Hz can be achieved for every target concentrations (Fig. 2). As shown in Fig. 2b, the target-binding process completes around 20 h, so 20 h is set as the standard condition for target incubation in this work. In the meantime, since the target concentration detected in this work is far below the critical concentration of spontaneous aggregation [27], the soluble oligomer will not aggregate and interfere with the target-binding process during the incubation.

3.4.2. Specific recognition of Aβ 1–42 soluble oligomer

Fig. 3 depicts the SWV responses at 5 Hz obtained upon analyzing a series of control experiments. As shown in Fig. 3a, in the presence of the control species, very little response change is observed, indicating that the special sequence recognized by the probe is indispensible. Furthermore, the monomer control produces a negligible response alteration comparable to that of the blank control, indicating the unrestrained molecular dynamics and the lack of defined secondary structure of the monomer (Fig. 3a).

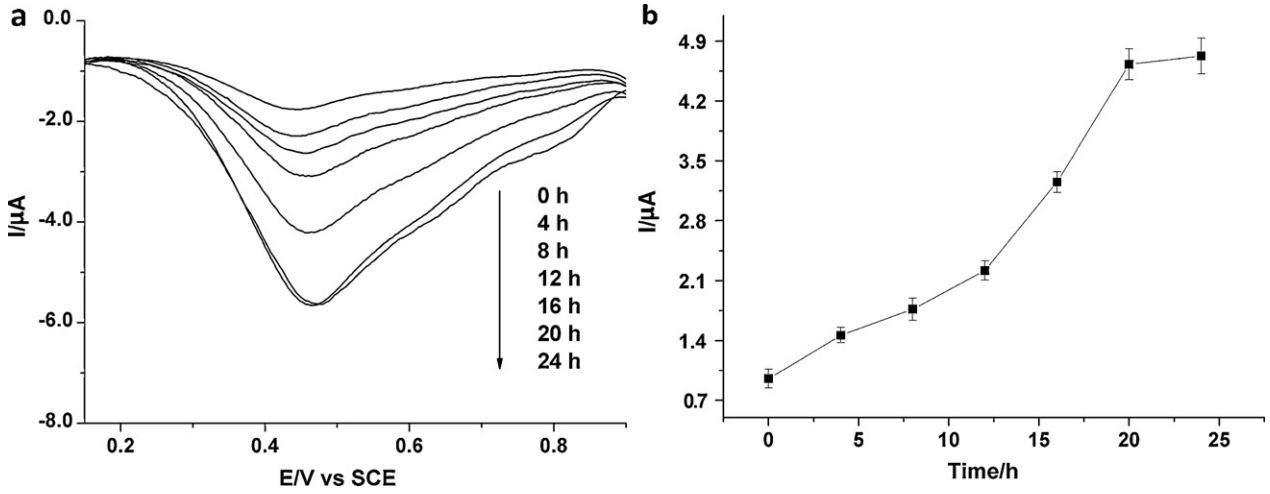


Fig. 2. (a) SWV responses at 5 Hz in 1 M $NaClO_4$ after the incubation of the sensor with a 10 mM pH 7.4 PBS solutin containing Aβ 1–42 soluble oligomer for 0 h, 4 h, 8 h, 16 h, 20 h and 24 h at room temperature, respectively. Target concentration (equivalent monomer): 12 nM. (b) Dependence of the increment of response on the incubation time. The error bars represent the standard deviation from the average ($n = 3$).

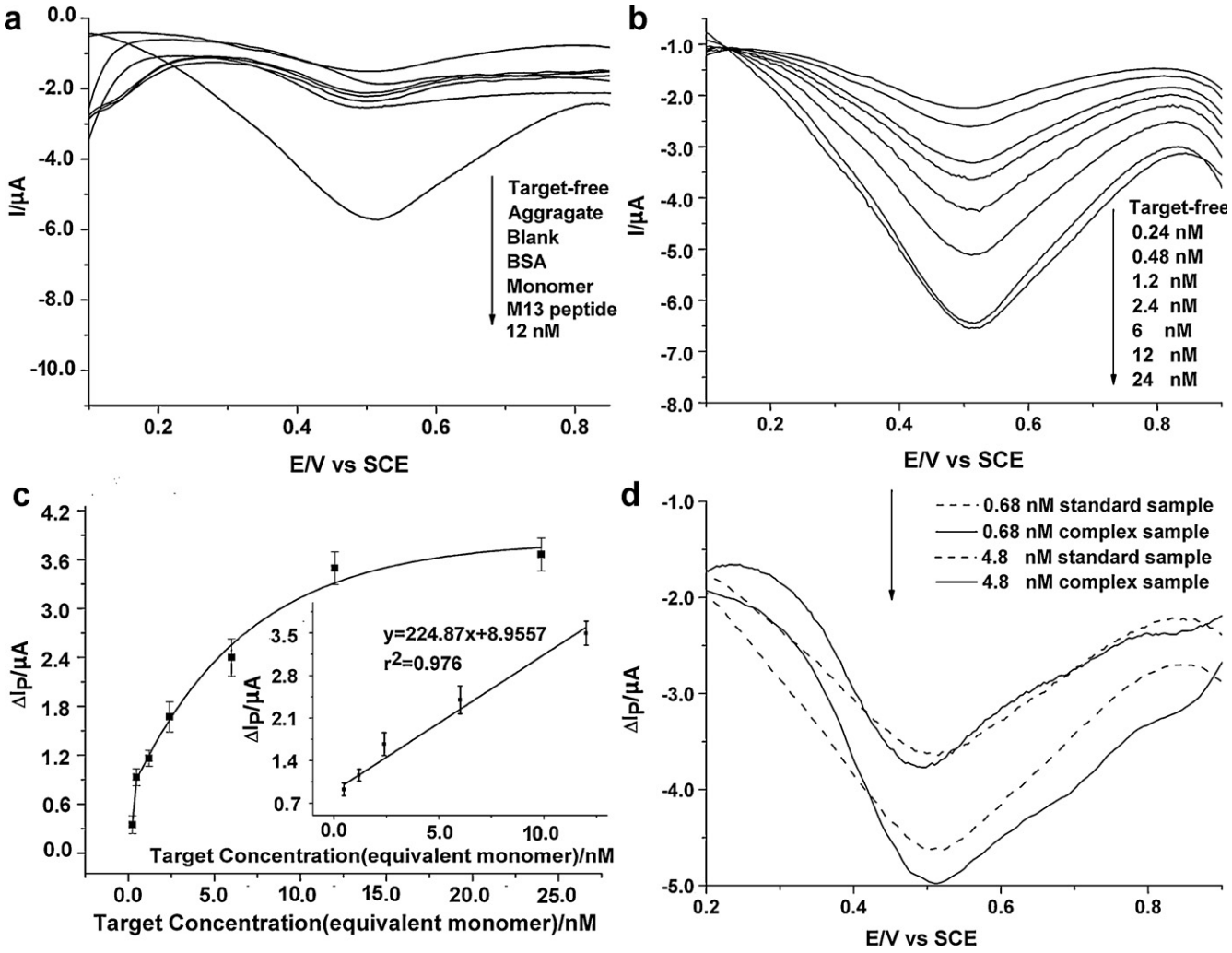


Fig. 3. (a) SWV responses for control experiments. (b) SWV responses for different concnetrations of target. (c) Dependence of the increment of response on the concentration of the soluble oligomer. ΔI_p, difference between the absolute value of peak response of target-bound sensor and that value of target-free sensor. Insert shows the linear range. The error bars represent the standard deviation from the average ($n = 3$). (d) SWV responses for pure sample (dashed line) and undiluted fetal bovine serum solutin containing Aβ 1–42 soluble oligomer (solid line). All the other conditions are the same as Fig. 1.

Table 1
Assay results of serum prepared samples by using the proposed biosensor and the comparison of our results with the added Aβ 1–42 soluble oligomer concentrations in serum samples.

Samples	Aβ 1–42 soluble oligomer concentration detected by proposed method (nM)	Added Aβ 1–42 soluble oligomer concentration (nM)	Relative error (%)
1	4.74	4.80	1.2
2	0.71	0.68	4.8

The aggregated target produces further repressed response, due to surface fouling induced by adsorption of concentrated fibrillar aggregate (Fig. 3a). These results confirm that the proposed peptide-based sensor may maintain conformation-specific recognition mechanism [24] and possess a good selectivity towards Aβ 1–42 soluble oligomer. We should mention that the insoluble species may not be a major obstacle to our sensor. Firstly, the fibrillar entanglement has such strong tendency to precipitate that the body fluid sample is unlikely to contain this species in large quantity comparable to our control. Secondly, if abundant fibrillar precipitate is detected, functional or even organic abnormality has developed in the patient, body fluid assay intended for early diagnosis is unnecessary in this scenario.

3.4.3. Quantitative determination of Aβ 1–42 soluble oligomer

For the detection of Aβ 1–42 soluble oligomer, the peptide-based electrochemical sensor is interrogated with targets of a series of concentrations (Fig. 3b). From the obtained results, we know that the SWV response increases with the concentration of the target, fulfilling our expectation that the synchronization phenomenon at 5 Hz becomes more prominent at higher concentrations as more probe molecules are stiffened by the captured soluble oligomer molecules. Target as low as 240 pM can still produce a fairly detectable signal-on response, indicating that this biosensor may have a good sensitivity. To figure out the dependence of the sensor response on target concentration, we have plotted the increment of peak response at 5 Hz as a function of the target concentration (Fig. 3c). Linear dependence can be obtained over the concentration range from 480 pM to 12 nM (Fig. 3c, inset).

We have further examined the feasibility of the developed biosensor by using several serum samples. Sensor responses from serum sample solutions prepared by dissolving different concentrations of soluble oligomer into undiluted fetal bovine serum have been recorded (Fig. 3d, solid line). The comparison between the assay results and the given concentrations in these serum samples has been shown in Table 1 and the relative errors are less than 5%.

4. Conclusion

We have investigated the interaction between peptide probe modified on an electrode surface and target on the basis of SWV synchronization with the surface ET of the modified electrode by using Aβ 1–42 soluble oligomer as a model. The change of surface ET upon target binding and the tune of the frequency of SWV have enabled us to prepare signal-on biosensors without complicated probe design. The quantitative experimental data also proves the SWV method suitable to follow interaction between the probe and Aβ 1–42 oligomer. This study may lay the groundwork for design of such kind of biosensor for other protein molecules, towards which short peptide ligands are at hand, such as EF-hand protein, Bax and HSP 70 [32–34].

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2012.02.055.

References

[1] K. Shiba, Curr. Opin. Biotechnol. 21 (2010) 412–425.
[2] V. Askoxylakis, R. Garcia-Boy, S. Rana, S. Kramer, U. Hebling, W. Mier, A. Altmann, A. Markert, J. Debus, U. Haberkorn, PloS One 5 (2010) 15962.
[3] M. Hall III, S. Misra, M. Chaudhuri, G. Chaudhuri, Microb. Pathog. 50 (2011) 252–262.
[4] T. Kobayashi, M. Kakui, T. Shibui, Y. Kitano, Mol. Biotechnol. 48 (2011) 147–155.
[5] S. Kanki, D.E. Jaalouk, S. Lee, A.Y. Yu, J. Gannon, R.T. Lee, J. Mol. Cell. Cardiol. 50 (2011) 841–848.
[6] F.C. Dudak, I.H. Boyaci, B.P. Orner, Molecules 16 (2011) 774–789.
[7] J.M. de la Fuente, C.C. Berry, Bioconjug. Chem. 16 (2005) 1176–1180.
[8] J. Gomez, S. Matsuyama, Methods Mol. Biol. 683 (2011) 465–471.
[9] A. Lowery, H. Onishko, D.E. Hallahan, Z. Han, J. Control. Release 150 (2011) 117–124.
[10] Z. Li, C. Cho, Curr. Pharm. Des. 16 (2010) 1180–1189.
[11] M. Baine, D.S. Georgie, E.Z. Shiferraw, T.P.T. Nguyen, L.A. Nogaj, D.A. Moffet, J. Pept. Sci. 15 (2009) 499–503.
[12] K. Chawla, T. Yu, S. Liao, Z. Guan, Biomacromolecules 12 (2011) 560–567.
[13] S. Laurenson, M.R. Pett, K. Hoppe-Seyler, C. Denk, F. Hoppe-Seyler, N. Coleman, P.K. Ferrigno, Anal. Biochem. 410 (2011) 161–170.
[14] Q. Song, L.J. Stadler, J. Peng, P.K. Ferrigno, Faraday Discuss. 149 (2011) 79–92.
[15] Y. Yang, L. Guo, N. Qu, M. Wei, L. Zhao, B. Wan, Biosens. Bioelectron. 28 (2011) 284–290.
[16] K. Kerman, H. Song, J.S. Duncan, D.W. Litchfield, H. Kraatz, Anal. Chem. 80 (2008) 9395–9401.
[17] A.M. Samoylov1, T.I. Samoylova, S.T. Pathirana, L.P. Globa, V.J. Vodyanoy, J. Mol. Recognit. 15 (2002) 197–203.
[18] S.E. Creager, T.T. Wooster, Anal. Chem. 70 (1998) 4257–4263.
[19] M. Ohtani, Electrochem. Commun. 1 (1999) 488–492.
[20] G.A. Orlowski, Peptide Monolayers: An Electrochemical Study, University of Saskatchewan, 2007.
[21] R. Ikeda, S. Kobayashi, J. Chiba, M. Inouye, Chem. Eur. J. 15 (2009) 4822–4828.
[22] R.J. White, K.W. Plaxco, Anal. Chem. 82 (2010) 73–76.
[23] D. Walsh, I. Klyubin, J. Fadeeva, M. Rowan, D. Selkoe, Biochem. Soc. Trans. 30 (2002) 552–556.
[24] T. Sato, P. Kienlen-Campard, M. Ahmed, W. Liu, H.L. Li, J.I. Elliott, S. Aimoto, S.N. Constantinescu, J.N. Octave, S.O. Smith, Biochemistry 45 (2006) 5503–5516.
[25] C. Hultschig, H. Hecht, R. Frank, J. Mol. Biol. 343 (2004) 559–568.
[26] M. Gobbi, F. Re, M. Canovi, M. Beeg, M. Gregori, S. Sesana, S. Sonnino, D. Brogioli, C. Musicanti, P. Gasco, Biomaterials 31 (2010) 6519–6529.
[27] Y. Liang, D.G. Lynn, K.M. Berland, J. Am. Chem. Soc. 132 (2010) 6306–6308.
[28] S. Komorskylovric, M. Lovric, Anal. Chim. Acta 305 (1995) 248–255.
[29] M. Lovric, D. Jadresko, Electrochim. Acta 55 (2010) 948–951.
[30] J.H. Reeves, S. Song, E.F. Bowden, Anal. Chem. 65 (1993) 683–688.
[31] S.L. Bernstein, N.F. Dupuis, N.D. Lazo, T. Wyttenbach, M.M. Condron, G. Bitan, D.B. Teplow, J.E. Shea, B.T. Ruotolo, C.V. Robinson, Nat. Chem. 1 (2009) 326.
[32] K.L. Yap, J.B. Ames, M.B. Swindells, M. Ikura, Proteins 37 (1999) 499–507.
[33] T. Yoshida, I. Tomioka, T. Nagahara, T. Holyst, M. Sawada, P. Hayes, V. Gama, M. Okuno, Y. Chen, Y. Abe, Biochem. Biophys. Res. Commun. 321 (2004) 961–966.
[34] A.L. Rérole, J. Gobbo, A. De Thonel, E.P. Schmitt, J.P. Pais de Barros, A. Hammann, D. Lanneau, E. Fourmaux, O. Deminov, O. Micheau, Cancer Res. 71 (2011) 484–495.