

Available online at www.sciencedirect.com**ScienceDirect**

Procedia Engineering 120 (2015) 41 – 44

**Procedia
Engineering**www.elsevier.com/locate/procedia

EUROSENSORS 2015

Characterization of Fibrillization Process of Amyloid-Beta on Lipid Membrane Utilizing a Cantilever-Based Liposome Biosensor

Z. Zhang^{a*}, M. Sohgawa^b, K. Yamashita^a, M. Noda^a^a*Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan*^b*Niigata University, 8050 Ikarashi 2-no-cho, Nishi-ku, Niigata 950-2181, Japan*

Abstract

We have developed a strain-gauge cantilever-based biosensor with liposomes as model cell membrane immobilized on the cantilever surface to investigate time course of dynamic behavior of amyloid-beta (1-40) protein ($A\beta(1-40)$) on lipid membrane. The results of this work clearly exhibit the characteristic of chronological change of the gauge resistance, which is closely related to the dependence of $A\beta$ -liposome interaction extent on the state of $A\beta$ during fibrillization and the type of lipid molecule. Therefore, it is considered that these results can adequately reflect the fibrillization process of $A\beta(1-40)$ on different kinds of lipid membranes. Meanwhile, it means that we are able to discriminate the molecular states of $A\beta$ and evaluate $A\beta$ fibrillization process using the cantilever-based liposome biosensor.

© 2015 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Peer-review under responsibility of the organizing committee of EUROSENSORS 2015

Keywords: Micro-cantilever; Droplet-sealing structure; Resistance change rate; Liposome; Amyloid-Beta; Fibrillization; Interaction.

1. Introduction

It is generally believed that amyloid- β protein ($A\beta$), which exists in fibrillar forms as a major component of senile plaques, is central to the development of Alzheimer's disease (AD) [1,2]. According to the amyloid hypothesis of AD, pathologic accumulation of $A\beta$ fibrils on cell membranes triggers neuronal death in the AD patient's brain. Therefore, the study on the process and characteristics of $A\beta$ fibrillization on cell membrane is very important to the

* Corresponding author. Tel.: +81 80 4501 6066; fax: +81 75 724 7400.

E-mail address: zhang-zy@kit.ac.jp

pathogenesis elucidation and early diagnosis approach of AD. However, as it is difficult to directly observe the dynamic behavior of A β on brain cells, detection of amyloid growth on cell membrane has been proved to be a challenging task. Recently we have reported that our fabricated NiCr-strain-gauge cantilever-based biosensor, where liposomes as sensing biomolecules are immobilized on the cantilever surface, is effective for evaluation of concentration and species of target proteins (e.g. CAB, lysozyme) [3]. Here we use liposome as model cell membrane and focus on applying our cantilever-based liposome biosensor to characterize the fibrillization process of A β on the lipid membrane. Note that, at present, there are few literatures reporting label-free evaluation of A β using micro-cantilever. On the other hand, the 40- and 42-amino acid forms of A β proteins, namely A β (1-40) and A β (1-42), respectively, are two major species found in brains of AD patients [4]. This time real-time investigation of dynamic behavior of A β (1-40) on lipid membrane becomes the discussion topic.

2. Experimental procedure

The micro-cantilever with the NiCr thin film strain gauge was fabricated as described previously [3]. This time 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) were selected as the lipid molecules to prepare two types of liposomes. The preparation of liposome and the immobilization of liposome on the cantilever surface were carried out in the reported manner [3]. It is noted that we further improved the airtightness of the droplet-sealing structure by filling the seams between the cover and reservoir with undried polydimethylsiloxane (PDMS), leading to the maintenance of stable liquid condition of target solution for more than 21 h. Fig. 1 shows the schematic cross section of the improved droplet-sealing structure adhered on the cantilever chip.

Fig. 2 illustrates the measurement procedure. Non-aggregated A β (1-40) powder was dissolved in ammonia water (0.1 wt%) to prepare 10- μ M A β (1-40) solution. Then the non-aggregated A β (1-40) solution was directly introduced onto the sensor, and the fibrillization process of A β on the DPPC or POPC lipid membrane was investigated through monitoring the resistance change of the strain gauge embedded in cantilever for 21 h. The resistance change rate of the strain gauge, $\Delta R/R_0$, is proportional to the deflection of the cantilever [5], which is caused by liposome-A β interaction. In $\Delta R/R_0$, R_0 represents the initial resistance (approximately 4.5 k Ω) of the strain gauge, and ΔR represents the change of resistance with time.

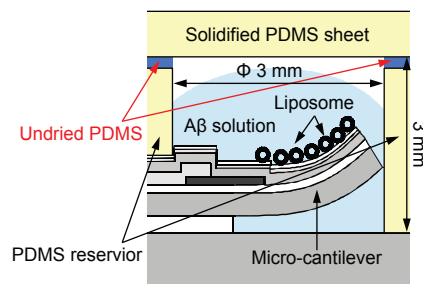


Fig. 1. A cross-sectional view of the cantilever-based liposome biosensor with the improved droplet-sealing structure.

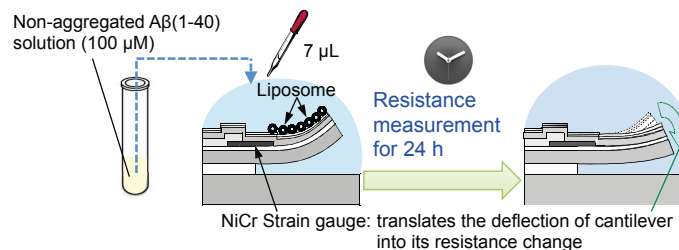


Fig. 2. Procedure for investigating A β fibrillization on liposome using the cantilever-based liposome biosensor.

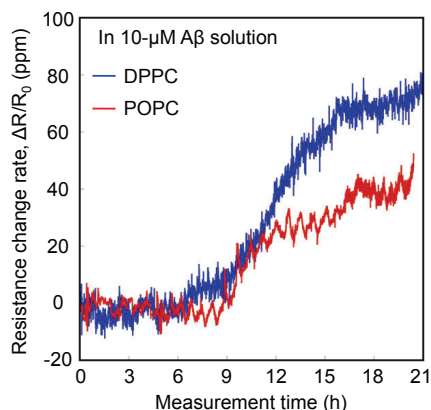


Fig. 3. Resistance change rates of the strain gauges embedded in DPPC-liposome-immobilized cantilever and POPC-liposome-immobilized cantilever in 10- μ M A β (1-40) solutions as a function of measurement time.

3. Results and discussion

Fig. 3 exhibits the resistance change caused by liposome-A β interaction during the process of A β fibrillization on the lipid membrane. It is clear from Fig. 3 that, regardless of the type of liposome (DPPC or POPC) immobilized on the cantilever surface, the resistance remains unchanged for only 8 h in the A β (1-40) solution. After 8 h measurement, the resistance significantly increases with time until it reaches relative steady-state after measuring for about 16 h. As we know, A β monomers do not interact with lipid membrane and aggregation of A β enhances its interaction ability [6]. It is also well known that A β aggregation-intermediates are much more toxic to cell membrane than A β fibrils themselves [6,7]. Combined with the experimental results, the characteristics of A β (1-40) during its fibrillization on lipid membrane are summarized. During the period of 0-8 h, A β is mainly in monomeric state and has very low interaction ability to liposome to induce the surface stress change of the cantilever. However, with the rapid aggregation of A β from 8 to 16 h, the liposome-A β interaction occurs on a large scale and A β fibrils grow on liposomes, which cause the increasing deflection of cantilever. Then the liposome-A β interaction is weakened by complete fibrillization of A β , and the resistance change rate reaches relative steady-state state.

On the other hand, the highest value of the resistance change rate measured by DPPC-liposome-immobilized cantilever sensor (about 70 ppm) is higher than that measured by POPC-liposome-immobilized cantilever sensor (about 40 ppm). Kotarek *et al.* has reported that liposomes with a greater degree of carbon saturation (e.g., DPPC liposome) better support the growth of bound A β (1-40) aggregation-intermediates on lipid membrane than liposomes with low saturation (e.g., POPC liposome) [8]. The result obtained in this work is in good agreement with Kotarek's conclusion.

Based on the above results, it is clear that the A β aggregation/fibrillization process on lipid membrane can be monitored in real time using the cantilever-based liposome biosensor, which would contribute to the technology development of A β label-free detection and the mechanism elucidation of A β fibrillization on cell membrane.

4. Conclusion

A very long-time stable measurement in A β solution is achieved owing to the improvement in the airtightness of droplet-sealing structure. As the resistance change reflects the extent of liposome-A β interaction, it is found that the fibrillization process of A β (1-40) are almost the same between on DPPC liposome and on POPC liposome, but the extent of liposome-A β interaction at a stage of A β fibrillization process is dependent on the type of lipid molecule. The results obtained in this work indicate that the employed cantilever-based liposome biosensor facilitates the characterization of A β fibrillization process, which offers a possibility for label-free detection of A β .

Acknowledgements

This research was supported in part by a Grant-in-Aid for Scientific Research (KAKENHI Grant No. 25249048 and 26630157) from the Japan Society for the Promotion of Science (JSPS).

References

- [1] D.J. Selkoe, Alzheimer's disease: a central role for amyloid, *J. Neuropathol. Exp. Neurol.* 53 (1994) 438-447.
- [2] J. Hardy, D.J. Selkoe, The amyloid hypothesis of Alzheimer's disease: progress and problem on the road to therapeutics, *Science* 297 (2002) 353-356.
- [3] Z. Zhang, T. Akai, M. Sohigawa, K. Takada, K. Yamashita, M. Noda, Bioassay of proteins in stable solution state using a novel cantilever-based liposome biosensor, in: *Proc. IEEE Sens. 2014*, IEEE Press, NJ, 2014, pp. 317-320.
- [4] S.A. Gravinam, L.B. Ho, C.B. Eckman, K.E. Long, L. Otvos, L.H. Younkin, N. Suzuki, S.G. Younkin, Amyloid β protein ($A\beta$) in Alzheimer's disease brain: Biochemical and immunocytochemical analysis with antibodies specific for forms ending at $A\beta_{40}$ or $A\beta_{42(43)}$, *J. Biol. Chem.* 270 (1995) 7013-7016.
- [5] C.H. Schwalb, C. Grimm, M. Baranowski, R. Sachser, F. Porrati, H. Reith, P. Das, J. Müller, F. Völklein, A. Kaya, M. Huth, A tunable strain sensor using nanogranular metals, *Sensors* 10 (2010) 9847-9856.
- [6] K. Matsuzaki, Physicochemical interactions of amyloid β -peptide with lipid bilayers, *Biochim. Biophys. Acta* 1768 (2007) 1935-1942.
- [7] H.A. Lashuel, D. Hartley, B.M. Petre, T. Walz, P.T. Lansbury Jr, Neurodegenerative disease: amyloid pores from pathogenic mutations. *Nature* 418 (2002) 291.
- [8] J.A. Kotarek, M.A. Moss, Impact of phospholipid bilayer saturation on amyloid- β protein aggregation intermediate growth: A quartz crystal microbalance analysis, *Anal. Biochem.* 339 (2010) 30-38.