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

Z. Zhang\textsuperscript{a*}, M. Sohgawa\textsuperscript{b}, K. Yamashita\textsuperscript{a}, M. Noda\textsuperscript{a}

\textsuperscript{a}Kyoto Institute of Technology, Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan
\textsuperscript{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\textsubscript{\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\textsubscript{\beta}-liposome interaction extent on the state of A\textsubscript{\beta} during fibrillization and the type of lipid molecule. Therefore, it is considered that these results can adequately reflect the fibrillization process of A\textsubscript{\beta}(1-40) on different kinds of lipid membranes. Meanwhile, it means that we are able to discriminate the molecular states of A\textsubscript{\beta} and evaluate A\textsubscript{\beta} fibrillization process using the cantilever-based liposome biosensor.

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

1. Introduction

It is generally believed that amyloid-\beta protein (A\textsubscript{\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\textsubscript{\beta} fibrils on cell membranes triggers neuronal death in the AD patient’s brain. Therefore, the study on the process and characteristics of A\textsubscript{\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 Aβ 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 Aβ on the lipid membrane. Note that, at present, there are few literatures reporting label-free evaluation of Aβ using micro-cantilever. On the other hand, the 40- and 42-amino acid forms of Aβ proteins, namely Aβ(1-40) and Aβ(1-42), respectively, are two major species found in brains of AD patients [4]. This time real-time investigation of dynamic behavior of Aβ(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 Aβ(1-40) powder was dissolved in ammonia water (0.1 wt%) to prepare 10-μM Aβ(1-40) solution. Then the non-aggregated Aβ(1-40) solution was directly introduced onto the sensor, and the fibrillization process of Aβ 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, ΔR/R₀, is proportional to the deflection of the cantilever [5], which is caused by liposome-Aβ interaction. In ΔR/R₀, R₀ represents the initial resistance (approximately 4.5 kΩ) 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.](image1)

![Fig. 2. Procedure for investigating Aβ fibrillization on liposome using the cantilever-based liposome biosensor.](image2)
3. Results and discussion

Fig. 3 exhibits the resistance change caused by liposome-Aβ interaction during the process of Aβ 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 Aβ(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, Aβ monomers do not interact with lipid membrane and aggregation of Aβ enhances its interaction ability [6]. It is also well known that Aβ aggregation-intermediates are much more toxic to cell membrane than Aβ fibrils themselves [6,7]. Combined with the experimental results, the characteristics of Aβ(1-40) during its fibrillization on lipid membrane are summarized. During the period of 0-8 h, Aβ 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 Aβ from 8 to 16 h, the liposome-Aβ interaction occurs on a large scale and Aβ fibrils grow on liposomes, which cause the increasing deflection of cantilever. Then the liposome-Aβ interaction is weakened by complete fibrillization of Aβ, 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 Aβ(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 Aβ 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 Aβ label-free detection and the mechanism elucidation of Aβ fibrillization on cell membrane.

4. Conclusion

A very long-time stable measurement in Aβ solution is achieved owing to the improvement in the airtightness of droplet-sealing structure. As the resistance change reflects the extent of liposome-Aβ interaction, it is found that the fibrillization process of Aβ(1-40) are almost the same between on DPPC liposome and on POPC liposome, but the extent of liposome-Aβ interaction at a stage of Aβ 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 Aβ fibrillization process, which offers a possibility for label-free detection of Aβ.
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