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A Novel Detection Method of Biomarker Molecule of α-Synuclein for Parkinson Disease by Liposome-Immobilized Cantilever Biosensor Using Self-Templating Phenomena of Prionoid Protein

Ryoko Kobayashi[1], Masanori Sawamura[2], Hodaka Yamakado[2], Masayuki Sohgawa[3] and Minoru Noda[1]

[1] Kyoto Institute of Technology
Matsugasaki, Sakyo-ku, Kyoto 606-8585, Japan
Phone: +81-75-724-7436 E-mail: m9621026@edu.kit.ac.jp
[2] Kyoto University
54 Syogoin-Kawaharacyo, Sakyo-ku, Kyoto 606-8507, Japan
Phone: +81-75-751-3767
[3] Niigata University
8050 Ikarashi 2-no-cho, Nishi-ku, Niigata 950-2181, Japan
Phone: +81-25-262-7819

Abstract

We newly examined a self-templating effect of prionoid protein on a liposome-immobilized cantilever sensor in order to detect a trace amount of α-synuclein fibril as biomarker molecule for Parkinson Disease. The developed sensor can significantly decrease the detection threshold down to several hundreds of fM and its measuring time to a few hours, and could be applied in a clinical setting.

1. Introduction

Parkinson Disease (PD) is second common neurodegenerative disorder, but its pathogenesis mechanism is still unclear and the treatment for PD has also not been well established at the moment. α-synuclein (aSyn) has been majorly recognized as a causative molecule for PD. Its monomer starts to aggregate and grow to toxic form [1] such as oligomers, protofibrils and fibrils in a pathological condition (Fig. 1). For the early diagnosis of PD, it is completely essential to detect the small amount of aggregated forms of aSyn in patient's cerebrospinal fluid (CSF). Additionally, it was revealed that aggregation of the monomeric aSyn is significantly promoted when a trace amount of aSyn fibril is mixed with the monomeric aSyn and quaked in a solution. Based on this phenomenon, real-time quaking-induced conversion (RT-QuIC) method [2,3] was developed and reported to be able to detect trace amount of aSyn aggregates in CSF by amplification of self-templating prionoid protein. However, RT-QuIC needs a process of several tens of thousands times shaking and measuring time as long as about 100 hrs.

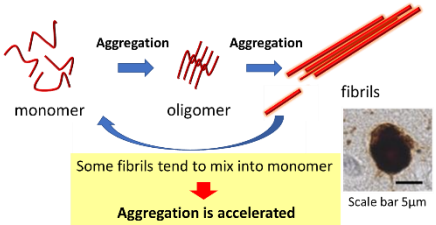

Fig. 1 An illustration of aggregation of aSyn from monomer to final fibril with a surface photo of aSyn fibrils on human brain cell.

In this work, we newly applied a liposome-immobilized cantilever sensor on an SOI wafer via Si MEMS processes [4], where the liposome would detect aSyn aggregates promoted by the addition of aSyn monomers by its self-templating effect.

2. Experimental Methods

A. AFM observation of aSyn fibrillization on liposome

Before electrical measurements of the developed microsensor, we checked fibrillized aSyn on liposome immobilized with cantilever surface by AFM in liquid.

B. Detection by liposome-immobilized cantilever sensor

First, we basically started to observe and detect the chronological phenomena of aggregation of aSyn fibril after adding it into phosphate buffered saline (PBS) solvent. As a reference, PBS itself without aSyn addition was preliminarily measured whether it would not show the other indispensable chronological change in resistive change in NiCr gauge of the cantilever. Thereafter, fibrillar aSyn was introduced and measured as a function of concentration.

Next, different from the above experiment, a high-concentrated (7 μM) aSyn monomer solution was set into the reservoir. Thereafter, low-concentrated aSyn (down to 700 fM) fibril was measured. Before and during the injection step, both extraction and injection of the mixed solution was repeated a few times through syringe needle (Fig. 2). This is completely different from RT-QuIC method that needs several tens of thousands of shaking of the target solution.

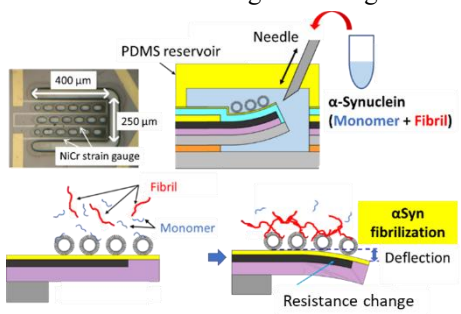

Fig. 2 Detection of aSyn on the liposome-immobilized cantilever sensor by using self-templating phenomena of prionoid protein.

3. Results and Discussion

First, the chronological behavior and interaction of αSyn with liposomes immobilized on the surface of the cantilever was examined by AFM in liquid (Fig. 3). From the figure, several aggregates as large as about 20-50 nm was observed on the liposome after the addition of αSyn. Also, the increased height from about 9 to 12 nm suggests the existence of grown aSyn fibrils.

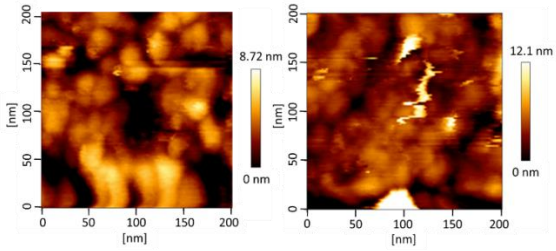

Fig. 3 AFM images of liposomes immobilized on Au surface of cantilever sensor (left: in PBS, right: after 40 min from addition of αSyn (monomer 35 μM, fibril 70 μM) in PBS)

Second, we measured chronological behaviors of aSyn in fibrillar states by our developed liposome-immobilized cantilever sensor system. Figure 4 shows time courses of change rate of gauge resistance of cantilever sensor for recombinant aSyn fibril in PBS solvent (70, 7 nM, 700, 70, 7, 0 pM). As a reference, firstly, PBS solvent without aSyn addition was observed to show negligible chronological change as in Fig. 4(a). We could measure several concentrations of recombinant aSyn in fibrillar state. Successfully, the aSyn aggregates in PBS were detected, compared to CSF of healthy person who has only monomeric aSyn not aggregated (not shown). Note that aSyn was detected down to 7 pM. From the figure, it is found that both resistance change rate and the transit time until the abrupt increase in resistance increase monotonously with increase in added aSyn fibril concentration. These results are considered to be reasonable because the interaction between the fibrillar aSyn and phospholipid and also association reaction would be larger for high-concentrated aSyn in the solution.

Next, Fig. 4(b) shows time courses of change rate of gauge resistance of cantilever sensor for recombinant aSyn fibril (70, 7, 0.7, 0 pM) added in 7 μM monomeric aSyn, respectively. This time we measured less than pM order, as mild cognitive impairment (MCI) of PD would have around 1 pM aSyn multimer from oligomer to fibril.

As seen from Fig. 4(b), chronological change is negligible for only monomeric aSyn (7 μM) without addition of fibrillar aSyn, indicating little interaction between monomeric aSyn and phospholipid of DPPC used. After adding the fibrillar aSyn, the tendency of time course became similar to Fig. 4(a); both resistance change rate and the transit time until the increase in resistance increase monotonously with increase in added aSyn fibril concentration.

As a result, the sensitivity was successfully improved up to 700 fM by mixing monomer and fibrillar aSyn, based on the self-templating effect of prionoid protein. This result revealed that the obtained sensitivity has become the same level as that of practically-used ELISA in clinical medicine.

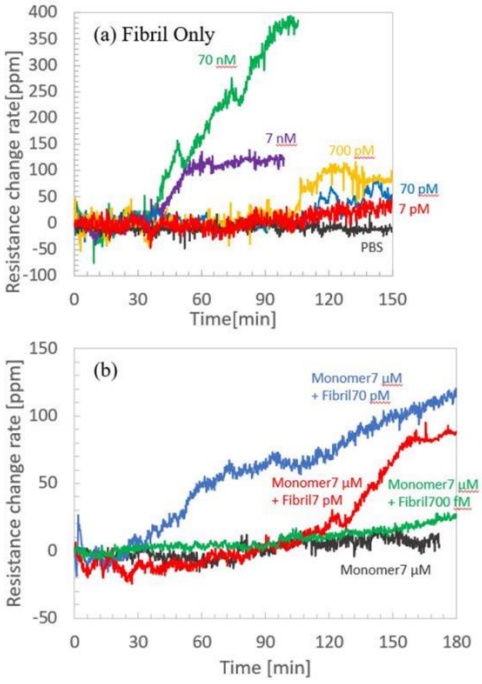

Fig. 4 Time courses of change rate of gauge resistance of cantilever sensor (a): for recombinant aSyn fibril (70, 7 nM, 700, 70, 7, 0 pM) in PBS solvent, (b): for recombinant aSyn fibril (70, 7, 0.7, 0 pM) added in 7 μM aSyn monomer.

4. Conclusions

We newly examined a self-templating effect of prionoid protein on a liposome-immobilized cantilever sensor in order to detect a trace amount of aSyn fibril as biomarker molecule for PD. It was revealed that the sensor using the self-templating effect can significantly enhance the sensitivity down to several hundreds of fM detection. The detection level has been reached to those of practically used ELISA.

Acknowledgements

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