Biosensors and Bioelectronics ■ (■■■■) ■■■–■■■

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Sensitive electrical detection of human prion proteins using field effect transistor biosensor with dual-ligand binding amplification

Shofarul Wustoni [a], Sho Hideshima [b], Shigeki Kuroiwa [b], Takuya Nakanishi [b], Masahiro Hashimoto [b], Yasuro Mori [b], Tetsuya Osaka [a,b,*]

[a] Graduate School of Advanced Science and Engineering, Waseda University, 3-4-1 Okubo, Shinjuku-ku, Tokyo 169-8555, Japan
[b] Institute for Nanoscience & Nanotechnology, Waseda University, 513 Waseda-tsurumaki-cho, Shinjuku-ku, Tokyo 162-0041, Japan

ARTICLE INFO

Article history:
Received 6 June 2014
Received in revised form
8 August 2014
Accepted 8 August 2014

Keywords:
Field effect transistor
Thiamine
Blood serum
Prion protein
Early diagnosis
Metal ion

ABSTRACT

Simple and accurate detection of prion proteins in biological samples is of utmost importance in recent years. In this study, we developed a label-free electrical detection-based field effect transistor (FET) biosensor using thiamine as a probe molecule for a non-invasive and specific test of human prion protein detection. We found that thiamine-immobilized FETs can be used to observe the prion protein oligomer, and might be a significant test for the early diagnosis of prion-related diseases. The thiamine-immobilized FET was also demonstrated for the detection of prion proteins in blood serum without any complex pre-treatments. Furthermore, we designed a dual-ligand binding approach by the addition of metal ions as a second ligand to bind with the adsorbed prion protein on the thiamine-immobilized surface. When the prion attached to metal ions, the additional positive charge was induced on the gate surface of the FET. This approach was capable of amplifying the magnitude of the FET response and of enhancing the sensitivity of the FET biosensor. Detection of prion proteins has achieved the required concentration for clinical diagnosis in blood serum, which is less than 2 nM. In summary, this FET biosensor was successfully applied to prion detection and proved useful as a simple, fast, sensitive and low-cost method towards a mass-scale and routine blood screening-based test.

1. Introduction

Prion proteins that can conformationally change into the aggregate form have played a vital role in several infectious and harmful prion diseases, such as the Creutzfeldt–Jakob disease and transmissible spongiform encephalopathies (TSEs). Until now, prion diseases were recognized as highly contagious and scarcely incurable (Prusiner, 1991; Collins et al., 2004; Mays et al., 2014). These diseases can easily transfer among different species by contaminated food from an infected animal and by blood transfusion from a preclinically infected individual (Yang et al., 2005; Liang et al., 2013). Such diseases have become of major concern and a critical health problem; in fact, some countries including Australia, Japan and the USA have established a nationwide surveillance system of human prion diseases (Ladogana et al., 2005; De Pedro-Cuesta et al., 2006). Thus, development of an analytical method to detect the presence of prion proteins in biological body fluid is of great importance to prevent further negative impacts of these diseases.

Currently, protein misfolding cyclic amplification (PMCA) is one of the most popular, powerful and commonly-used methods in practical tests for prion proteins and diseases (Saborio et al., 2001; Castilla et al., 2005; Chen et al., 2010; Moudjou et al., 2013). However, PMCA involves multiple steps including incubation, proteolysis and replication. These processes are time and labor consuming, suggesting that these methods are not appropriate for the use of mass-scale and routine blood screening-based tests. Over the past few years, numerous methods have been proposed to detect prion proteins, such as micromechanical resonator arrays (Varshney et al., 2009), immune-quantitative real-time PCR assays (Reuter et al., 2009), and quantum dot (QD) nanoparticles (Liang et al., 2013). Though such research demonstrated a good biosensing performance for prion detection, nevertheless most of them required complex functionalization and had technical limitations; for instance, those proposing micromechanical arrays and PCR required a labeling step and expensive instrumentation, while QD nanoparticles may have suffered difficulties when produced in stable synthesis and integrated into a miniaturized system. These issues highlight the urgency of the field and a continuous need to construct a simple, robust, rapid and low-cost detection method

* Corresponding author at: Waseda University, Okubo 3-4-1, Shinjuku-ku, Tokyo 169-8555, Japan. Tel.: +81 3 5286 3202; fax: +81 3 3205 2074.
E-mail address: osakatets@waseda.jp (T. Osaka).

http://dx.doi.org/10.1016/j.bios.2014.08.028
0956-5663/

that can diagnose prion proteins with a specific and sensitive approach.

We have attempted to develop field effect transistor (FET) biosensors as a promising biosensing device that provides a low-cost and label-free electrical detection for a decade. The FET offers a type of electrical biosensor that is operated by field-effect modulation of carriers in a semiconductor substrate due to adsorbed molecules with an electric charge on the gate electrode (Schoning and Poghossian, 2006; Hideshima et al., 2012). The charged molecule that specifically interacts with a probe molecule immobilized on the gate surface of the FET device can be the target (analyte) molecule. Recently, the FET biosensor has been intensively performed for promising diagnostic applications and exhibits a remarkable performance, including detection of DNA hybridization reactions (Fu and Li, 2010), antibody–antigen reactions (Hideshima et al., 2012), glycan–protein interactions (Hideshima et al., 2013) and cell identification (Chen et al., 2011). This biosensing system offers a simple, label-free, rapid and direct electrical detection of biomolecular interactions. Furthermore, fabrication of the FET biosensor has been well established through semiconductor technology, meaning that it is suitable for mass and low-cost devices as future point-of-care technology.

To capture prion proteins, we used thiamine as a small-sized probe molecule that possesses a specific interaction with prion proteins (Perez-Pineiro et al., 2011). The thiamine molecule, which is a smaller affinity ligand than using the whole monoclonal antibody, efficiently overcomes the limitation of the FETs' performance caused by the Debye screening length. This method is in accordance with the research trend in the field of biosensors by using a small molecule, such as an aptamer (Maehashi et al., 2007), antigen binding fragment (Elnathan et al., 2012), and glycan (Hideshima et al., 2013), as a probe. Recently, we employed Congo red as a probe interacting with a cross-β structure of amyloid fibrils for the detection of a prion-inducing fragment of the yeast protein Sup35 (Sup35NM) as well as an aggregation of the amyloid β (Aβ) protein by the FET (Hideshima et al., 2014a, 2014b). Here, thiamine, which has benefits as a stable, low-cost and easy-handling reagent, was used to detect the prion protein oligomer. Such small probe molecules might provide more advantages than antibodies or enzymes towards mass fabrication of biosensor devices for practical application.

By using the thiamine-immobilized FET, we can observe the existence of the prion protein oligomer. The oligomeric forms of prions were considered to be a key state to identifying and elucidating the conformational transition of prion proteins (Solowski et al., 2003). Other research on prion diseases confirmed that the prion protein oligomer has a critical toxicity and plays a potential role in early neuronal damage (Kayed et al., 2003; Walsh and Selkoe, 2004; Simoneau et al., 2007). Those theories indicated a crucial issue in developing a technique that is able to monitor the oligomeric form of prion proteins. Hence, the thiamine-immobilized FET, which is capable of detecting the prion protein oligomer, can provide early diagnosis of the molecular states of prion molecules with a simple electrical detection.

In addition, to achieve a highly-sensitive detection of prion proteins, we also applied a signal amplification method by using a dual-ligand binding mechanism between the prion protein, thiamine and metal ion. Dual-ligand bindings are formed by a sandwich-interaction with the prion protein between thiamine and metal ion. At first, the prion protein adsorbed onto the thiamine-immobilized gate surface of the FET. Then, the addition of the metal ion subsequently attached onto the specific binding-site in the prion protein. As a result, an increase of the surface charge density on the gate surface at the solid–liquid interface was generated due to the additional positive charge of metal ions. This approach enables the FET biosensor to extend the sensitivity into the desired limit of detection (LOD) of prion proteins in blood serum.

In the present study, we demonstrate that the thiamine-immobilized surface can be successfully used as a biosensing platform for the detection of prion proteins in human blood serum. The thiamine-immobilized FET biosensor can also be used to observe the prion protein oligomer, which is an important stage of the preclinical period of the disease. Then, the quantitative performance of the FET biosensor for prion proteins in the blood serum sample was examined by the addition of metal ions via the dual-ligand binding approach.

2. Materials and methods

2.1. Reagents

The recombinant human prion protein, PrP^c (23-231), was purchased from Jena Bioscience (Germany). The prion protein was initially solubilized in 1.5 M of guanidine HCl and stored at −60 °C as a stock solution. Then, the prion protein sample solution was prepared by diluting the stock solution in a 10 mM phosphate buffer saline (PBS), with a pH of 7.4 (of at least 10-fold), followed by a pre-treatment using an ultrasonic chamber and vortex mixer. A fresh sample solution was used for each analysis of the electric measurement. Thiamine hydrochloride was purchased from Sigma-Aldrich and dissolved in 10 mM of PBS (pH 7.4). A self-assembled monolayer reagent, 3-aminopropyltriethoxysilane (APTES), and a crosslinker reagent, glutaraldehyde (GA), were purchased from Sigma-Aldrich. The organic solutions were provided by Kanto Chemical Co. Human serum albumin (HSA) and human serum type AB (male) were also purchased from Sigma-Aldrich. The chemicals of the metal ion (Copper Sulfate, $CuSO_4$; Manganese (II) Chloride, $MnCl_2$; Zinc Chloride, $ZnCl_2$) were purchased from Sigma-Aldrich and diluted using ultrapure water. A 10 mM PBS was made in-house using NaCl (137 mM), $Na_2HPO_4 \cdot 12H_2O$ (8.1 mm), KCl (2.7 mm), and KH_2PO_4 (1.5 mm), then 0.01 mM PBS for electrolytes on the electric measurement, prepared by diluting the 10 mM PBS with ultrapure water (Milli-Q, Millipore).

2.2. Preparation of thiamine-immobilized FET biosensor

A schematic image of the thiamine-immobilized FET biosensor is shown in Fig. 1. The n-type FET device, where the SiO_2 insulator layer on the Si substrate acted as the gate electrode with a size of 10 μm in length and 1000 μm in width, was fabricated by Toppan Printing Co. Ltd. (Japan). The FET devices were first cleaned with acetone and soaked for 10 min in an ultrasonic chamber to remove the layer of photoresist on the chip. The fabrication process for tailoring the nano-bio-molecular structure on the FET gate surface can be described as follows. First, the FET device was exposed with O_2 plasma at 200 W for 1 min (gas plasma device, model: PR301, Yamato Scientific Co., Japan), producing a hyrodxyl group on the gate surface. The gate surface was then incubated in a toluene solution containing a 1% (v/w) APTES reagent at 60 °C inside a glove box with an Ar atmosphere (99.99% pure). The APS-modified FET was ultrasonically cleaned in a mixture solution of methanol and toluene (1:1), then dried with a N_2 gas spray to remove the residue. The FET device was then annealed at 160 °C for 1 h.

Before electric measurement, the FET gate surface was immersed for 30 min at room temperature in PBS containing 2.5% GA, followed by washing with PBS. The fabrication of stable GA-modified aminopropylsilyl by reducing the Schiff bases was performed in the same way as the previous procedure

Please cite this article as: Wustoni, S., et al., Biosensors and Bioelectronics (2014), http://dx.doi.org/10.1016/j.bios.2014.08.028