Effect of Amyloid Conformation on the Response of Field Effect Transistor Biosensor to Sup35NM Protein

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Detection of amyloid proteins is of great significance in finding Prion diseases such as scrapie, bovine spongiform encephalopathy (BSE) and Creutzfeldt-Jakob disease. These diseases are very threatening and common affected to the human beings in the field of neurodegenerative disorders. Established methods of detecting amyloid proteins including stain polarimetry and immunoassay techniques such as enzyme-linked immunosorbent assay (ELISA) require cost and time-consuming sample preparation.

Field effect transistor (FET) devices, which are easily manufactured through the semiconductor processes, have considerable promise as electronic transducers for rapid and sensitive detection of biorecognition events [1-4]. The detection process does not require labeling; therefore, their successful applications in medical fields have been expected because of the simplicity and rapidity. The FET detects potential change on the gate surface by the intrinsic charge of target molecules which react with probe molecules immobilized on the gate surface as threshold voltage shift (ΔV_g) of gate voltage (V_g)-drain current (I_d) characteristics. Here we propose the use of the FET biosensor in detection of a kind of amyloid fibrils, Sup35NM, which is known as a good model to be used in amyloid formation. We applied congo red (CR), which is a representative molecule interacting with Sup35NM, as a probe and demonstrated that the CR-immobilized surface of the FET gate is successfully utilized to detect Sup35NM (Figure 1).

To test the specificity of the CR-immobilized surface of the FETs, we evaluated the response of FET in the presence of Sup35NM or human serum albumin (HSA) solutions. The target molecule of Sup35NM interest here is in the form of amyloid fibril (after incubation for 3-4 days) and the concentration is 1.2 μM, for which the specific interaction between CR and amyloid fibril is expected. Here the Sup35NM is considered to have negative charge due to the measurement condition (pH = 7.4) because its isoelectric point (pI) is calculated equal to 4.9 from amino acid sequences. As shown in Figure 2a, the ΔV_g of CR-immobilized FET in the positive direction was observed equal to around 36 mV when the target protein Sup35NM was added. On the other hand, little shift was observed when a non-related protein HSA (pI = 4.7) was added (Figure 2b) as a negative control, suggesting that few HSA molecule adsorbed on the gate surface. The results suggest that the CR-immobilized FET possesses the specificity to detect amyloid proteins. Furthermore, we examined the response of the CR-immobilized FET to various forms of Sup35NM. The Sup35NM changes its conformation depending on incubation time. Interestingly, the FET response to Sup35NM depends on its amyloid conformation, of which detail will be discussed at the conference.

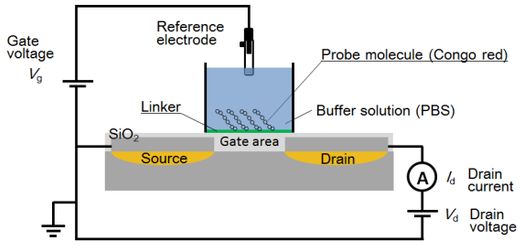

Figure 1 Schematic illustration of congo red (CR)-immobilized field effect transistor biosensor for the detection of amyloid Sup35NM.

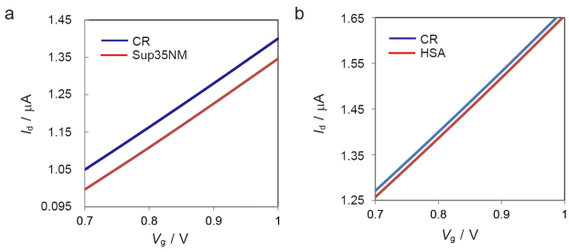

Figure 2 Voltage shifts of V_g–I_d curve caused by addition of two kinds of proteins, (a) Sup35NM and (b) HSA, onto CR-immobilized FETs.

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