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Affinity sensor for haemoglobin A1c based on single-walled carbon nanotube field-effect transistor and fructosyl amino acid binding protein

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ABSTRACT

Haemoglobin A1c (HbA1c) is a significant glycaemic marker for diabetes mellitus. The level of HbA1c reflects the mean blood glucose level over the prior 2–3 months and it is useful for the assessment of therapeutic effectiveness and for diagnosis. In this study, we report the label-free affinity sensor for HbA1c based on the chemiresistor-type field-effect transistor, which has a simple sensor configuration. Single-walled carbon nanotubes (SWNTs) were used as the transducing element. The fructosyl amino acid binding protein from *Rhizobium radiobacter* (SocA), which binds to α-fructosyl amino acid specifically, was used as the biorecognition element for fructosyl valine (FV), the product of the proteolytic hydrolysis of HbA1c. The developed sensor shows the ability to measure as low as 1.2 nM FV, which is 14-fold more sensitive compared to the previously reported fluorescence-based sensor using SocA. This sensor also exhibits high specificity where no significant response is observed from either fructosyl lysine (FK) or glucose, which are potential interferents. FK is the ε-fructosyl amino acid from glycated albumin, another glycated protein, whereas glucose is naturally present at very high concentration in the blood. We propose that the modulation of the surface charges on the SWNTs caused by the conformational change in SocA upon ligand binding leads to the proportionate changes in the number of carriers in the SWNT channel.

1. Introduction

Haemoglobin A1c (HbA1c) is an important glycaemic marker for diabetes mellitus. It is a product of the non-enzymatic reaction between glucose and the amine group of the β-subunit N-terminal valine residue of haemoglobin. The level of HbA1c reflects the mean blood glucose level over the prior 2–3 months and it is not affected by the daily fluctuations in the blood glucose concentration caused by diet, stress, or activity (Bunn et al., 1978; Franco, 2012; Goldstein et al., 2004). Currently, the level of HbA1c is measured primarily by ion-exchange chromatography, boronate affinity chromatography, immunoassay, and enzymatic assay at clinical laboratories. While these methods are highly accurate, they are performed generally in centralised laboratories with large and expensive instruments; therefore, they are time-consuming, expensive, and require complicated analytical procedures. Commercialised point-of-care testing devices are available for HbA1c based on boronate affinity separation or immunoassay. However, the majority of devices are low in accuracy compared to clinical laboratory methods (Hirst Jennifer et al., 2017). Therefore, the development of portable HbA1c testing devices with high accuracy and simple operation is required.

Previously, we reported the fructosyl amino acid binding protein from *Rhizobium radiobacter* (SocA) (Sakaguchi et al., 2005). This protein is a bacterial periplasmic protein undergoing significant conformational changes by the binding of ligands. SocA has the molecular weight of 28.8 kDa and was found to bind to α-fructosyl amino acid specifically. We therefore developed a fluorescent sensing system using SocA for the measurement of fructosyl valine (FV), which is the α-fructosyl amino acid yielded by the proteolytic hydrolysis of HbA1c. We created the cysteine-introduced mutant of SocA to modify the environmentally

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sensitive fluorophore acrylodan at the specific site. The developed analytical protocol could measure as low as 17 nM FV which is over 100-fold more sensitive than enzymatic detections (Sakaguchi et al., 2007). Therefore, this indicates that a highly sensitive affinity-based sensor for HbA1c could be developed using SocA. Considering fluorescence-based measurements require a light source and light detector, electrochemical sensing system will be preferable for handheld sensing system. However, no electrochemical device was reported using SocA as the molecular recognition element.

The chemiresistor-type field-effect transistor (FET) have previously been used for label-free affinity-based biosensing with a very simple configuration. This sensor comprises the source and drain electrodes, and a semiconducting channel between the electrodes thereof. As this chemiresistor-type FET does not require a gate electrode, unlike the traditional FET-based biosensor, the configuration of this biosensor is very simple. Here, any charged biomolecules near the semiconducting material provide the electrostatic gating effects. Based on this mechanism, we can observe the modulation of the number of charge carriers in the channel caused by the affinity-based binding between the recognition element and the target molecules on semiconducting channel. Semiconducting single-walled carbon nanotubes (SWNTs) were used as the semiconducting channel of this device. SWNT can be considered a one-dimensional allotrope of carbon, and can be described as a ribbon of graphene comprising sp^2 hybridised carbon atoms seamlessly rolled into a cylindrical tube. The SWNT is a p-type semiconductor and can be used as a semiconducting channel of a FET because of its high surface-area-to-volume ratio and it can be easily functionalised with biorecognition molecules (Tran and Mulchandani, 2016). Therefore, using the SWNT as a transducing element, a highly sensitive chemiresistor-type FET-based biosensor can be developed for various biomarkers using aptamers, antibodies, or other proteins as the biorecognition elements (Cella et al., 2010; Das et al., 2011; Park et al., 2010; Rajesh et al., 2016; Ramnani et al., 2013; Tlili et al., 2011).

In this study, our objective is to develop a label-free affinity-based electrical sensor for HbA1c by combining the SWNT-FET and SocA as the biorecognition element for FV, the proteolytic product of HbA1c which has been used in the enzymatic HbA1c diagnostic kit. The schematic of developed biosensor is shown in Fig. 1. The configuration of the developed sensor is very simple, as it comprises the source and drain electrodes and SWNT as the channel element between them. The SocA bioreceptor is immobilized on the semiconducting SWNT channel. Upon the binding of FV, SocA undergoes a conformational change on the SWNT channel. We have developed a highly sensitive sensor for FV with a very simple configuration and procedure compared to the previously reported fluorescence-based measurement using SocA. Furthermore, we also showed that our biosensor is specific to FV and is not affected by either fructosyl lysine (FK) or glucose, which are potential interferents. FK is the ε-fructosyl amino acid from glycated albumin

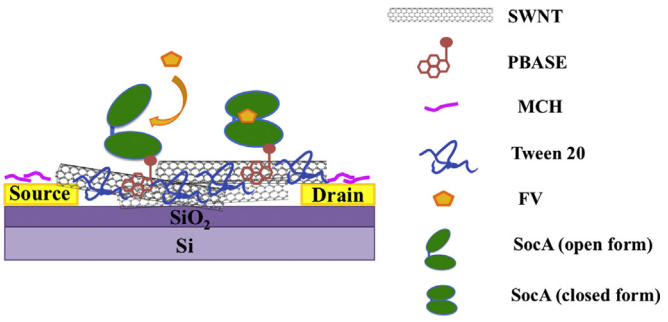

Fig. 1. Schematic of biosensor in this study. The sensor comprises the source and drain electrodes and a SWNT channel between the electrodes thereof. SocA was functionalised on the SWNTs using PBASE. The exposed gold region of the source and drain electrodes was blocked with MCH and SWNT was blocked with Tween 20. SocA undergoes the conformational change by binding of FV.

(GA), another glycated protein used as a glycaemic control marker for diabetes. Glucose is naturally occuring molecule present in very high concentration in the blood. We proposed a sesning mechanism for this biosensor where the modulation of the charges on the surface of the SWNTs caused by the conformational change in SocA upon ligand binding leads to the change in the number of charge carriers in the SWNT channel.

2. Materials and methods

2.1. Materials

SocA was prepared recombinantly according to Sakaguchi et al., (2005). After preparation of purified SocA protein, the binding ability of prepared SocA to FV was checked with autofluorescence measurement before using in FET biosensor. The details of preparation method and binding ability evaluation of SocA are shown in Supplementary Information (S1). FV was prepared as previously described (Keil et al., 1985). N^{α}-carbobenzyloxy-N^{ε}-fructosyllysine (Z-FK) was prepared following the method by Hashiba (1976). The semiconducting SWNT solution was acquired from NanoIntegris (Boisbriand, Quebec, Canada). (3-Aminopropyl)triethoxysilane (APTES), 6-mercapto-1-hexanol (MCH), 1-pyrenebutanoic acid succinimidyl ester (PBASE), ethanolamine (EA), and Tween 20 were acquired from Sigma-Aldrich (St. Louis, MO, USA). The electrical measurements were performed using the Keithley 2636 System Source Meter® (Tektronix, Beaverton, OR, USA).

2.2. Fabrication of the biosensor

The sensor chip with the patterned electrodes (five pairs of source and drain electrodes with 10 μm width separated by 10 μm (The schematic of the structure of the sensor chip is shown in Fig. S2)) on a Si/SiO_2 substrate was incubated with APTES for 1 h, followed by the dropcasting of the SWNT solution (the average nanotube length is approximately 0.7 μm with tubes ranging from 100 nm to 4 μm according to the data sheet from NanoIntegris). After drying the SWNT solution at room temperature overnight, the excess SWNTs were rinsed with DI water and isopropyl alcohol (IPA), and subsequently annealed at 250 °C in air for 1 h to obtain pure SWNTs between the source and drain electrodes. The exposed gold was blocked with MCH by immersion in 50 mM MCH dissolved in ethanol for 1 h followed by washing with IPA and drying with N_2 gas.

SocA was immobilized on the SWNT by the following method: the SWNT-functionalised chip was submerged in 3 mg/mL of PBASE dissolved in DMF for 1 h. After washing with DMF and drying with N_2 gas, 500 μg/mL of SocA in 10 mM phosphate buffer (PB) pH 7.0 was deposited on the SWNT-functionalised region of the chip and incubated at 4 °C for 2.5 h. Subsequently, excess PBASE was blocked with ethanolamine (EA) by depositing 100 mM EA in 10 mM PB pH 7.0 and incubating for 1 h at 4 °C. SWNT was blocked by incubating 1% Tween 20 on the SWNT-functionalised region of the chip at 4 °C for 1 h. Subsequently, the chip was washed with 10 mM PB pH 7.0.

The detail of the device fabrication is described in Supplementary Information (S2). The characterization of prepared sensor is also described in Supplementary Information (S3).

2.3. Sample measurement using prepared biosensor

The device resistance (R) was measured in real-time to meaure the response to different concentration of FV by applying a steady potential of V_{SD} = 0.1 V. Initially, 40 μL of 10 mM PB pH 7.0 was deposited on the sensing region of the biosensor. Subsequently, various concentrations of small volumes (1–4 μL) of FV were added in the PB on the biosensor to yield a final FV concentration of 0, 1.2, 13, 55, 97, 481, 1010, 1483, and 1909 nM.

To evaluate the specificity of the prepared biosensor, responses to Z-

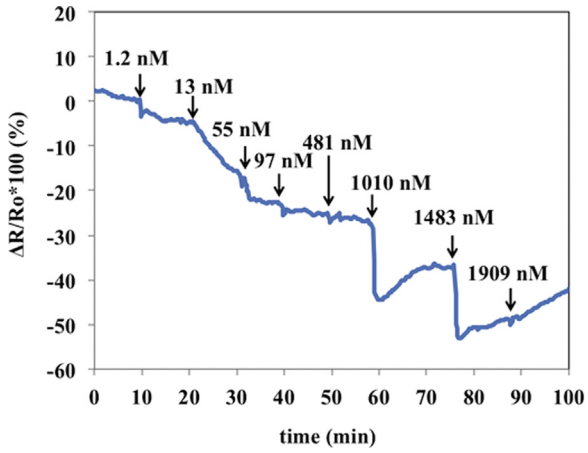

Fig. 2. Response curve of fructosyl valine (FV) measurement by applying V_{SD} = 0.1 V. The Y-axis is the normalised % resistance change calculated as $(R-R_0)/R_0*100 = \Delta R/R_0*100$, where R_0 is the resistance before FV addition, and R is the resistance after FV addition. Each arrow shows an FV addition point and the concentration value shows the final concentration at that point.

FK and glucose were measured using the same method as the FV measurement.

3. Results

3.1. Measurement of fructosyl valine

A representative response curve of the SocA-functionalised biosensor to increasing concentrations of FV is shown in Fig. 2. The Y-axis is the normalised % resistance change calculated as $(R-R_0)/R_0*100 = \Delta R/R_0*100$, where R_0 is the resistance before the addition of FV, and R is the resistance after the addition of FV. Each arrow shows an FV addition point and the concentration values show the final concentrations at that point. As shown in the response curve, the resistance dropped after the addition of FV solution into the PB on the sensing region of the biosensor, and reached to the steady value around 10–15 min after the FV addition. While the resistance was getting to be the steady state, the binding between the FV and the SocA became the equilibrium state, and also, the solution reached to homogeneous after mixing the high concentration of FV solution into the buffer solution on the device.

The calibration curve plotting the normalised % resistance change against the FV concentration is shown in Fig. 3 (red square) (n = 3). The result of the FV measurement using the biosensor without SocA is also shown in Fig. 3 (blue circle) (n = 3). A decrease in the resistance according to the FV concentration from 1.2 nM to 1500 nM was observed with the SocA-immobilised biosensor, while no change was observed in the resistance with the biosensor without SocA immobilization. Therefore, it was confirmed that the prepared biosensor could measure the various concentrations of FV successfully only when SocA was immobilized on SWNT as the molecular recognition element.

3.2. Evaluation of the specificity of the biosensor

To evaluate the specificity of developed biosensor, the potential interferents, Z-FK and glucose, were assayed. Z-FK is the synthetic substrate of ε-FK, which are the proteolytic product derived from GA, the other glycated protein used as a glycaemic control marker for diabetes as HbA1c. While FV is glycated at α-amine group of valine residue, ε-FK is glycated at ε-amine group of lysine residue and in Z-FK, α-amine group of lysine is blocked with benzyloxycarbonyl group. The structures of FV, ε-FK and Z-FK are shown in Fig. 4. Glucose was also tested with our biosensor because it is contained in blood at mM level,

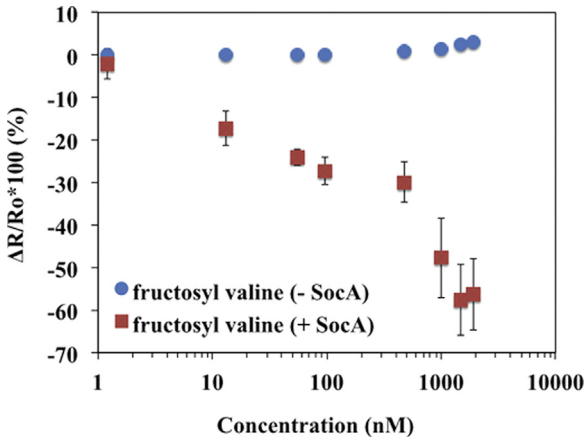

Fig. 3. Calibration curves plotting normalised % resistance change against the FV concentration. Each plot shows the result of FV measurement with the SocA-functionalised sensor (square), and FV measurement with SocA non-functionalised sensor (circle).

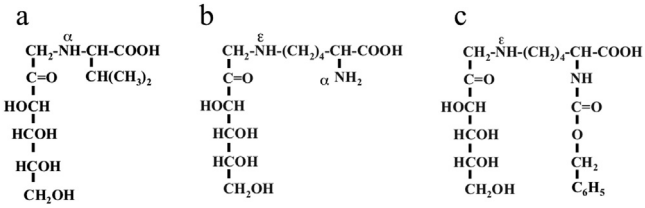

Fig. 4. Structures of (a) FV, (b) ε-FK, and (c) Z-FK. FV is glycated at α-amine group of valine residue, ε-FK is glycated at ε-amine group of lysine residue and in Z-FK, which is the synthetic substrate of ε-FK, α-amine group of lysine is blocked with benzyloxycarbonyl group.

which is very high concentration (≧125 mg/dL (6.9 mM) of fasting blood glucose and ≧200 mg/dL (11 mM) of random blood sugar for diabetes). Z-FK and glucose were tested from 1.2 to 1909 nM and from 9.8 nM to 53 mM, respectively with the SocA-immobilised biosensor. The normalised % resistance changes by 1 μM FV, 1 μM Z-FK, 1 μM glucose and 12 mM glucose are shown in Fig. 5 (n = 3). While a large change in sensor response was observed with 1 μM FV (48%), small

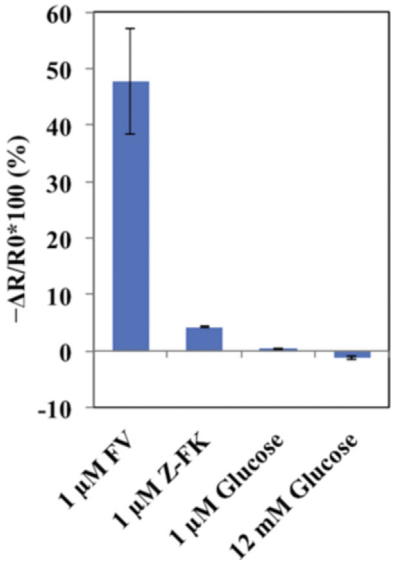

Fig. 5. Evaluation of the specificity of the developed biosensor. The minus normalised % resistance change obtained with 1 μM FV, 1 μM Z-FK, 1 μM glucose and 12 mM glucose are shown.

changes in sensor responses were observed with 1 μM Z-FK (4.3%) and 1 μM glucose (0.35%), and even with high concentration of 12 mM glucose (-1.3%). The normalised % resistance changes by the Z-FK and glucose measurements were less than 4.5% and 4%, respectively, over the entire concentration range (Calibration curves of Z-FK and glucose measurements are shown in Supplementary Information with Fig. S2–1 and Fig. S2–2). From these results, it was suggested that the developed biosensor is specific to FV and not affected by either Z-FK or glucose.

4. Discussion

In this study, we developed a label-free affinity sensor by combining the chemiresistor-type SWNT-FET, which has very simple configuration, and fructosyl amino acid binding protein for α-fructosyl amino acid, SocA, for the application in HbA1c measurement. In this sensor, the electrostatic gating effects are provided by charged biomolecules near the semiconducting channel. Therefore, we can observe the modulation of the number of carriers in the channel caused by the affinity-based binding between biorecognition element and the target molecules by functionalization of the semiconducting element with biorecognition element for the target molecule, and avoiding the any non-specific binding to the semiconducting element by proper blocking procedure. SWNT was used as the semiconducting transducing element in this study. The SWNT is a p-type semiconducting material and is a promising transducing element because of its high surface-area-to-volume ratio. Our SWNT dropcasting method results in a uniform and dense network of 1–3 layers of (predominantly single-layered) randomly oriented SWNTs bridging the source and drain electrodes on the Si/SiO_2 substrate, which has been observed in our previous study (Tan et al., 2015). On the basis of the SEM observation, we roughly estimated the number of SWNTs on the FET device. The analysis revealed that about 120 SWNTs were deposited per μm^2 ($1.2 \times 10^{14}/m^2$). Since the electrode area where SWNTs were deposited, was 12.56×10^{-6} m^2 (circle of diameter = 4 mm), it is estimated that 1.5×10^9 SWNTs were deposited per FET device. Considering the area and gap of gold electrode which compose single field-effect transistor was 10 μm x 10 μm (1.0×10^{-10} m^2) (Supplemental Information Fig. S2), 1.2×10^4 SWNTs were deposited per single sensor. In our previously published and present works and device characterization, we have ascertained that the adapted SWNT deposition method has yielded biosensors with the desired performance and sensitive responses. Here the randomly oriented SWNT network was of adequate density to physically and electrically bridge the source and drain electrodes forming the semiconducting sensing channel. The nanotubes electrically bridge the source and drain electrodes mainly through the tube-tube junctions formed throughout the SWNT network.

Furthermore, it is easy to functionalise the SWNT with biomolecules. Here, we used PBASE to immobilise SocA onto SWNTs. The pyrenyl group of PBASE binds to SWNTs via π–π stacking while the succinimidyl ester group reacts with the amine groups on the biorecognition molecule by amide coupling. The preparation procedure of the sensor was simple. The fructosyl amino acid binding protein from *Rhizobium radiobacter*, SocA, which specifically binds to α-fructosyl amino acids, was used as a biorecognition element to measure FV which is the product of proteolytic hydrolysis of HbA1c. We used 500 μg/mL of SocA solution to functionalize the SWNT on the sensor chip. Previous recent works from our lab have suggested that our SocA concentration used were within similar concentration ranges (10^1 to $> 10^2$ μg/mL) to achieve the desired biosensing performance (Ramnani et al., 2013; Tan et al., 2015; Wasik et al., 2017, 2018). On the basis of aforementioned SEM analyses, the amount of SWNTs is estimated that 1.5×10^9 SWNTs were deposited per FET device. We estimated the amount of immobilized SocA by measuring the excess unbounded SocA in the solution after the incubation of 20 μL of 500 μg/mL SocA, and also by measuring the washed SocA solution after the first incubation. These investigations revealed that about 8% of SocA in the initial solution (20 μL of 500 μg/mL SocA) was immobilized on the surface of FET device. Therefore, the total SocA projected area is estimated to be 4.5×10^{-4} m^2, assuming one SocA molecule's projected area is 2.1×10^{-17} m^2, which is estimated by the 3D structure of SocA. Considering the estimated total surface area of SWNTs immobilized on the FET device is 4.6×10^{-6} m^2, which was assumed from the total number of estimated SWNT (1.5×10^9 SWNTs) and a single SWNT surface area (3.1×10^{-15} m^2), the amount of immobilized SocA might cover more than 100 times of the surface of SWNT immobilized on FET devise. These estimations indicated that the condition we employed already yielded the maximum efficiency in the immobilization of SocA on the sensor device.

SocA consists of two domains linked by a hinge region. The protein undergoes conformational changes upon ligand binding from the open form to the closed form where the two domains come close to each other. The prepared biosensor shows the ability to measure FV from 1.2 nM to 1500 nM only when SocA is functionalised on the SWNT, while no signal is observed without SocA (Fig. 3). This detection limit at 1.2 nM is lower compared to the fluorescence-based sensor using SocA that could measure FV from 17 nM, as previously reported (Sakaguchi et al., 2007). Therefore, by combining SWNT-FET and SocA, a highly sensitive label-free affinity sensor for HbA1c can be developed. This sensor requires the pre-treatment of HbA1c to get the FV because the SocA recognizes FV, the fructosyl amino acid, and does not recognize the intact HbA1c. Current enzymatic methods for HbA1c measurement in clinical laboratories use either fructosyl amino acid oxidase (FAOx) or fructosyl peptide oxidase (FPOx). Similar to the SocA biosensor reported here, these enzymatic methods also cannot function with intact HbA1c, thus, requiring sample pre-treatment via proteolytic hydrolysis of HbA1c. Therefore, it is possible to practically combine the pre-treatment procedure of proteolytic hydrolysis with our SocA biosensor. We also evaluated the specificity of this developed biosensor to potential interferents, Z-FK and glucose. Z-FK is a synthetic substrate of ε-FK, which is an ε-fructosyl amino acid obtained from the proteolytic hydrolysis of glycated albumin (GA). It is another glycated protein used as a glycaemic control marker for diabetes and reflects the mean blood glucose level over the last 2–3 weeks (Guthrow et al., 1979). GA is quantified as the ratio of GA concentration to the total albumin concentration and the normal range is from 11% to 16%. We further evaluated sensor response to glucose at a high concentration of 53 mM to simulate the typical concentration range in human blood, especially in diabetic patients. We confirmed that the prepared SocA-functionalised biosensor was not affected by either FK or glucose, and was specific to FV (Fig. 5).

The level of HbA1c is described as the ratio of HbA1c to the total haemoglobin concentration, and the levels less than 5.7%, 5.7–6.4%, and 6.5% or higher are the level for normal, prediabetes and diabetes, respectively, according to American Diabetes Association. The normal haemoglobin level in whole blood is 13.5–17.5 g/dL for male and 12.0–16.0 g/dL for female (Kratz et al., 2004) and the molecular weight of haemoglobin is 64.5 kDa. Therefore, the FV concentrations released by the proteolytic hydrolysis of HbA1c contained in the whole blood can be calculated as around 130 μM for 5.7% HbA1c and 150 μM for 6.5% HbA1c, assuming that the average haemoglobin concentration is 15 g/dL. ε-FK can be contained at the same level (tens to hundreds μM level) in the proteolytic hydrolysis product of the blood. Considering that the developed biosensor in this study could measure the FV from 1.2 nM to 1500 nM, this sensor is useful for the practical use of HbA1c testing. Furthermore, when HbA1c level is measured with our biosensor, the sample solution should be diluted by hundreds times, which results in the significant dilution of the possible interfering substances, consequently minimizing any interference effects. To confirm the performance and to simulate the measurement of the developed FET based biosensor in the presence of varieties of potential ingredient, we performed an experiment to evaluate the sensor performance in the diluted simulated body fluid (SBF). The prepared SBF was diluted 500 times

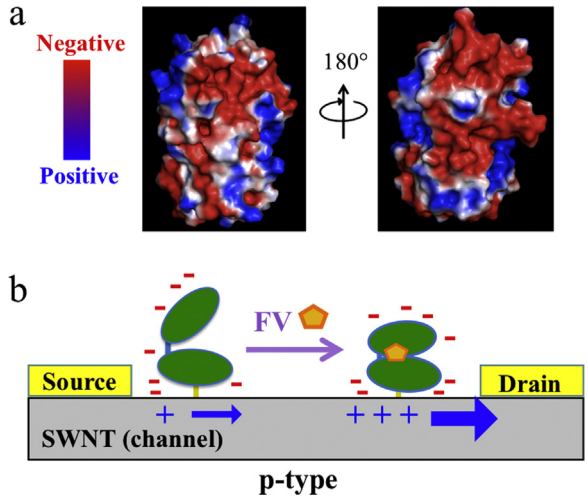

Fig. 6. (a) The evaluated charge distribution on the SocA surface. The red color and the blue color show the negative and positive charge, respectively. (b) Schematic for the proposed mechanism of the sensor in this study. On the semiconducting SWNT chennel, the SocA changes the structure from an open to closed form upon FV binding, which leads to the modulation of charges on the SWNT, thus affecting the number of carriers in the semiconducting channel.

using 10 mM PB pH7.0. The various concentrations of FV were measured with the same method as the FV measurement in 10 mM PB. Although the sensitivity was slightly decreased compared with the measurement in buffer solution, the decrease of the resistance depending on the FV concentration was observed and the measurement of FV in the target range was achieved (Supplementary Information S6). With these results, we expect the sensor performance in the practical sample in the presence of charged ingredient will be achieved by mandatory dilution of the sample due to the high sensitivity of the developed sensor.

Herein, we propose a mechanism that allows for the measurement of small FV molecules by our biosensor. As mentioned before, SocA changes its structure from the open to the closed form by binding the target molecules. Regarding the charge on the surface of this protein, the negative charge is dominant because the predicted isoelectric point of SocA (calculated using the ExPASy pI calculator online tool;ExPASy. Compute pI/Mw tool) is 6.5. Recently, the three-dimensional (3D) structure of SocA possessing the ligand (closed form) was elucidated (Marty et al., 2016). Based on this 3D structure, the surface charge of SocA was evaluated using software of Adaptive Poisson-Boltzmann Solver, as shown in Fig. 6a. This figure also shows that the negative charge is dominant on the surface of the SocA. While immobilized on the FET sensor, the conformational change in SocA that occurred by FV binding makes the protein compactly folded and increasing the density of negative surface charges, which leads to increase in the amount of hole carriers in the p-type SWNT channel (Fig. 6b). Consequently, the decrease in resistance was observed (Figs. 2 and 3).

Previously, Park et al. (2008) reported the monitoring of conformational changes of a maltose-binding protein (MBP) induced by maltose binding with an ion-sensitive field-effect transistor (ISFET). They immobilized MBP on the ISFET gate surface and detected the change in the drain current when MBP was exposed to the maltose. MBP also undergoes the conformational change with the maltose binding, and they mentioned that the changes in drain current could be explained with both “geometric effect” and “charge effect.” The geometric effect occurs when the MBP undergoes conformational change to the closed form, the area of MBP, which cover the surface of the sensor, decreases while the distance between the ISFET surface and MBP increases. The charge effect occurs when maltose binding occurs, a positively charged region in MBP moves away from the ISFET surface, which leads to a decrease in the strength of the electrical field between ISFET surface and MBP. The FV sensing mechanism of our chemiresistor-type FET biosensor using SocA undergoing conformational change is similar with the study using MBP by Park et al. (2008), even though the authors did not evaluate the dependence of the magnitude of the signal on the maltose concentrations. Although the potential of FET based biosensor utilizing the binding protein has been reported previously, this study is the first report of the development of a chemiresistor-type SWNT-based biosensor for quantitative measurement of the target ligand of the binding protein, SocA.

Further investigations to obtain the 3D structure of SocA without ligand (open form) can facilitate the comparison of the surface charges of SocA between the open and closed form, which may affect the magnitude of the FET signal. Additionally, we can optimise the orientation of SocA on the SWNT channel based on the structure investigation.

5. Conclusion

In this study, we presented a novel label-free affinity sensor for HbA1c based on a chemiresistor-type SWNT-FET using SocA as the biorecognition element for FV. The configuration of the sensor was very simple as it comprised the source and drain electrodes and a semiconducting SWNT channel between the electrodes thereof, which was functionalised with SocA. The developed biosensor showed the ability to measure as low as 1.2 nM FV. This is highly sensitive compared to the fluorescence-based sensing methods using SocA that we previously reported. Furthermore, this sensor was specific to FV and not affected by either FK, which came from GA, another glycated protein used as the glycaemic marker as HbA1c, or glucose, which is present in the blood at high concentrations. We also concluded that the high sensitivity of this biosensor was due to the unique character of SocA, in that it undergoes conformational changes upon ligand binding. The structural change from the open to closed form by FV binding could lead to the modulation of the number of carriers in the SWNT channel. This study is the first that reports the development of a chemiresistor-type SWNT-FET based biosensor for HbA1c that utilizes the fructosyl amino acid binding protein, SocA.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.bios.2018.09.069.

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