Single-Chained Fragment Variable (scFv) Recombinant as a Potential Receptor for SARS-CoV-2 Biosensor Based on Surface Plasmon Resonance (SPR)

Isa Anshori
Antonius Eko Nugroho
Aminul Solihin
Jessika Jessika
Department of Biomedical Engineering Institut Teknologi Bandung, Bandung, Indonesia
isaa@staff.stei.itb.ac.id

Muhammad Yusuf
Yeni Wahyuni Hartati
Silmina Prastriyati Sari
Taufik Ramdani Tohari
Research Center of Molecular Biotechnology and Informatics Universitas Padjajaran, Bandung, Indonesia
m.yusuf@unpad.ac.id

Brian Yuliarto
Gilang Gumilar
Department of Engineering Physics Institut Teknologi Bandung, Bandung, Indonesia
brian@tf.itb.ac.id

Lavita Nuraviana
Raih Rona Althof
Research Center for Nanoscience and Nanotechnology Institut Teknologi Bandung, Bandung, Indonesia
lavita.nuraviana@gmail.com

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***Abstract*— Detection of SASR-CoV-2 plays a significant role in reducing the transmission of COVID-19. Antigen swab test is widely used for screening due to its low processing time and cost, while RT-PCR is used in patient monitoring since it is quite expensive. Although the antigen swab test is more affordable than the RT-PCR, it only generates a discrete result: positive or negative. Thus, it cannot be used for patient monitoring. A method using antigen-antibody binding and surface plasmon resonance (SPR) principle was developed in this research to create an affordable, instant, and quantified SARS-CoV-2 detection method. In this study, modified scFv is tested as a potential bioreceptor since it is easier to be expressed than the whole antibody. The results show that the scFv with the best potential was harvested from the periplasm of *E. coli* and purified. It has a maximum response at 8.02 RU, LOD at 8.34 ng/mL, linearity at 1.38 in the range of 25-200 ng/mL, and a determination coefficient at 92 percent.**

Keywords: SARS-CoV-2; SPR biosensor; scFv; periplasmic; purification; binding characteristics.

I. Introduction

COVID-19 has been a global pandemic since March 2020. The detection method plays a crucial role in controlling this pandemic. A rapid test is used for screening due to its affordability and low processing time. However, it only generates a discrete result and cannot be used for other than screening. On the contrary, RT-PCR generates CT (Cycle Threshold) value which indicates the severity of the patient, thus it is used for patient's monitoring [1], [2] This method is quite expensive and needs so much time to get the result, minimum four hours by skilled analyst due to its complicated process, especially the RNA extraction, purification, and amplification [3], [4]. A simpler method as an alternative for RT-PCR is needed to reduce the cost and time of analysis.

The antibody-antigen principle is used in the antigen swab test to simplify the virus detection process. The immobilized recombinant antibody and gold nanoparticles complex will bind the target protein and changes the shape of nanoparticles, thus a certain color will be appeared on the cassette [5]. Instead of antibody, single-chained fragment variable (scFv) is used as the bioreceptor in this research because it is easier to be expressed than the whole antibody, thus it can reduce the cost [6], [7].

The binding activity of the scFv and the SARS-CoV-2 antigen will be measured using surface plasmon resonance (SPR). A similar method had been developed using localized surface plasmon resonance (LSPR) principle and RNA aptamer as the bioreceptor [8]. Since it detects the RNA of the virus, RNA extraction, purification, and amplification is needed [3]. Thus, this method still takes a lot of time and costs. Moreover, SPR is more preferred than LSPR in this research because the recent technology of the SPR technique allows automation and multiple measurement at the same time [9]. Besides, this technique also needs very small amount of sample since it uses microfluidic channel in most of the devices, and the potential of microfluidic in SARS-CoV-2 detection has been studied [10], [11]. Therefore, the developed biosensor can be illustrated in Figure 1.

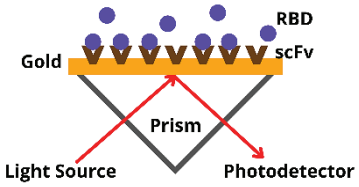

Fig. 1. Biosensor of SARS-CoV-2 using SPR technique and the scFv recombinant as the bioreceptor.

SPR is one of the commonly used techniques for binding analysis [12], [13] and has been used in the development of biosensors for various illnesses caused by bacteria and viruses. This technique was used in the development for H5N7 virus detection with a limit of detection (LOD) at 402/mL [14], dengue virus serotype 2 and 3 with LOD at $2x10^4$/mL [15], *Vibrio cholerae* with LOD at 10 CFU/mL [16], and *Salmonella typhimurium* with LOD at $2.1x10^6$ CFU/mL [17]. It is also used to detect various compounds in the human body, for example growth hormone (hGH) with LOD at 21.9 ng/mL [18].

SPR technique uses gold particles and a light source to create a plasmonic phenomenon. When the light source hits the gold particles at a certain angle, the energy from photons

is used to create a plasmon from the gold particle. Therefore, there is a difference between the amount of energy captured by the photodetector and the energy released by the light source [19], [20]. The photon-to-plasmon activity only happens when the momentum of the photon and the electron of gold particle are matched, thus the angle of the light source plays an important role [20], [21]. On the other hand, the momentum of the gold electron is influenced by the binding activity on the surface of the gold film. This binding activity will affect the change of permittivity on the film surface then change the critical angle [22]. When it is measured at a fixed angle, the change of critical angle leads to intensity changes.

Other than the ease of protein expression, the scFv has a lot of advantages. The scFv is formed by variable heavy (VH) and variable light (VL) connected by a peptide linker therefore has a similar performance to the whole antibody, although it has a tiny size [23], [24]. Besides, it is easier to be engineered to have the capability of binding the ACE2 receptor in the spike protein [25]. This advantage gives the scFv potential to be used as the bioreceptor of the SARS-CoV-2 biosensor.

The focus of this article is to prove the potential of the scFv CR3022 to be the bioreceptor for SARS-CoV-2 biosensor based on the SPR technique. The scFv CR3022 is the part of antibody produced by SARS patient from the outbreak in 2003 [26]. The binding site of scFv CR3022 was modified to have binding compatibility to SARS-CoV-2 RBD. Besides, a chain of cysteines was also added to the peptide linker to give the protein ability to be adsorbed to the gold surface. A biomolecular study of the design of scFv CR3022 was prepared for separate publication.

II. Materials and Methods

A. Chemicals and Apparatus

The modified scFv CR3022 was produced by Research Center of Molecular Biotechnology and Informatics Universitas Padjajaran. The SARS-CoV-2 RBD was purchased from Genscript, and the BSA was purchased from Sigma Aldrich. The ITM VitPAD from Universitas Padjajaran and PBS pH 7.4 from Sigma Aldrich were used for the sample storage media. The NanoSPR8 and the BA1080 plate by NanoSPR Devices was used as the SPR measurement device and the gold film plate since it has ability to measure eight samples at the same time.

B. Recombinant scFv Immobilization on Gold Surface

The gold plate assembly was done by placing the plate on a flat surface and dropping 25 µL of modified scFv CR3022 in Tris-Cl to the plate surface. Then, it was placed at room temperature for 90 minutes to make sure the scFv binds to the gold molecule. The plate was rinsed in PBS pH 7.4 to remove unbounded protein and the residue. Bovine serum albumin (BSA) 50 µL as a blocker was dropped to the plate surface and placed at room temperature for 15 minutes.

C. NanoSPR8 Configuration

Since the measurement environment is aqueous, prism FI-65-28 was used. The prism was placed on the holder. Then, about 20 µL of immersion oil was dropped and distributed evenly on the prism surface. An immobilized plate was placed on the prism followed by the microfluidic compartment. The angle of the measuring compartment was set to be around 58-69 degrees, and photodetectors were set for each measuring chamber. The continuous measurement was done in slope mode where the intensity changes are observed.

D. Biosensor System Analysis

There were two different sources of harvested scFv, the periplasm and the growth medium. Systems using the combination of those scFv were analyzed by measuring the binding activity of the SARS-CoV-2 RBD in various concentrations to the bioreceptor. The best scFv was purified and compared to the crude one.

III. Results and Discussion

A. Medium-Growth and Periplasmic scFv

There are two possible sources in the scFv harvesting, periplasm of *E. coli* and the growth medium. The protein is expressed in the periplasm. But, since the old parental cells were died and lysed, the scFv in the periplasm can be found in the growth medium [27]. Thus, proteins from different source were tested, and the result shows that periplasmic scFv is better than the medium-growth one, as shown in Figure 2.

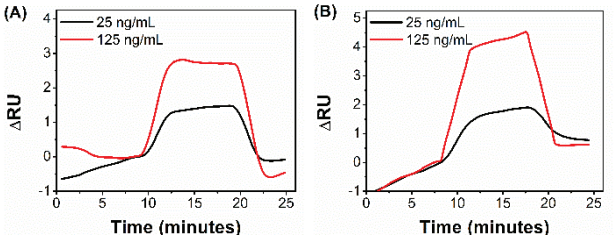

Fig. 2. Binding activity of 25 and 125 ng/mL SARS-CoV-2 RBD to: (a) medium-growth scFv; and (b) periplasmic scFv.

Periplasmic scFv has a higher response for 125 ng/mL SARS-CoV-2 RBD than the medium-growth scFv. This difference is caused by the defect on the medium-growth scFv. Since it is discharged to the growth medium which contains metabolic waste of the cells, the protein was damaged [28]. In certain cases, the defective scFv was still able to interact with the gold surface, but the antigen-binding region is broken. This protein decreases the maximum response in high concentrations, including 125 ng/mL.

B. Purification of Periplasmic scFv

The periplasmic scFv was purified and tested using SARS-CoV-2 RBD 125 ng/mL. As shown in Figure 3, the purified bioreceptor has a higher response. The purification leads to less impurity in the suspension, thus more protein can be immobilized to the gold SPR chip. More bioreceptor increases the binding activity therefore increases the SPR response [29], [30].

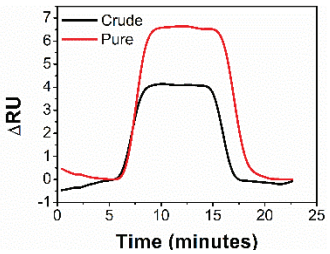

Fig. 3. Binding activity of 125 ng/mL SARS-CoV-2 RBD to crude and purified periplasmic scFv.

Normally, the suspension of scFv has impurity agents, including the native proteins [31]. Some native proteins also have the cysteine group. Therefore, the native proteins can interact with the gold surface during the immobilization process [32]. But these proteins will be flushed away during the washing procedure and will not influence the measurement. The reason why scFv cannot be flushed during the washing procedure is that it has not only a cysteine, but a chain of cysteines on its linker. Then, the existence of native proteins during the immobilization will reduce the number of scFv bound to the gold surface.

The binding characteristics, including the limit of detection (LOD), were obtained by measuring the response for different SARS-CoV-2 RBD concentrations and build the Langmuir Freundlich model for each system. It uses the Langmuir model in higher concentration and the Freundlich model in lower concentration. The adsorption model is based on Equation (1), meanwhile the linear model is based on Equation (3) [33]–[35]. The adsorption and linear model of purified periplasmic scFv are shown in Figure 4.

$$qE = (Qm(KS\ Ce)\text{^}mS) / (1+(KS\ Ce)\text{^}mS\ )) \quad (1)$$

$$K = Qm\ a = Qm\ (KS\text{^}mS\ ) \quad (2)$$

$$mS\ ln(Ce) - ln(a) = -ln(K/qe) \quad (3)$$

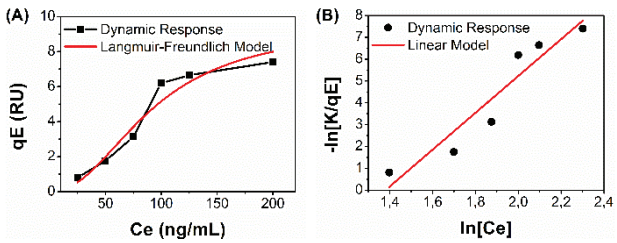

Fig. 4. (a) Adsorption model and (b) linear model, of purified periplasmic scFv using Langmuir-Freundlich model.

Beside the adsorption model, a non-linear fitting process also generates a value of maximum response (Qm), equilibrium constant (KS), and binding homogeneity (mS). These values are used to make the linear model which consists of ln(K/qE) versus ln(Ce) [35]. The outcomes of this process are the slope (m), the intercept (c) and its deviation (dev). Then, the detection limit can be calculated using Equation (4), derived from the Equation (3).

$$LOD = exp\text{^}(((-ln(K/(3.3)dev) - c))/m) \quad (4)$$

TABLE I. THE BINDING CHARACTERISTICS FOR PURIFIED PERIPLASMIC SCFV

Parameter	Definition	Value
Qm	Response capacity	8.02 RU
KS	Equilibrium constant	0.026
mS	Homogeneity	1.09
m	Slope of linear model	1.38
dev	Intercept deviation of linear model	0.49
LOD	Limit of detection	8.34 ng/mL
R^2	Determination coefficient	0.92

Based on Table I, the purified periplasmic scFv has a maximum response at 8.02 RU, adsorption equilibrium constant at 0.026, and homogenous binding since the mS is nearly one [33]. Moreover, the biosensor using this receptor has LOD at 8.34 ng/mL, linearity at 1.38 in the range of 25 – 200 ng/mL, and determination coefficient at 92 percent.

The developed biosensor was tested using RBD from another viruses. *Influenza-A virus* (AI) was chosen to represent viruses from different phylum, while *Avian coronavirus* (IB) was from different genus [36]. The result in Figure 5(a) shows that the developed system is specific enough for SARS-CoV-2 detection. The sensor was also stored in a freezer for different storage days. All sensor chips were immobilized in the same day but tested on different day. It shows that there is decrease of performance, and the performance was reduced to 80.1% in the third day. It is caused by the instability of the target binding site of the scFv [37]. Therefore, the sensor is best used in less than three days after the immobilization.

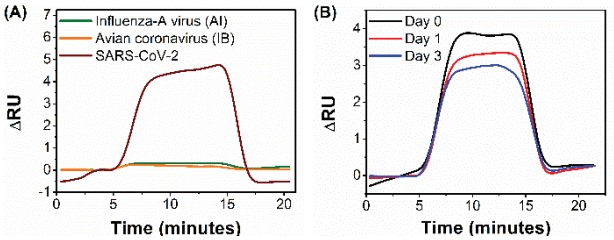

Fig. 5. (a) Control negative using other RBD and (b) stability of biosensor.

Since it is a preliminary study, the developed sensor has not been tested using real sample. Nasopharyngeal swab sample can contain as low as 6100 copies per mL or about 7.08 ng/mL of S protein where RBD exists [38], [39]. Meanwhile the developed sensor has the limit detection at 8.34 ng/mL. A further development should be done to decrease the detection limit. Therefore, it works well in monitoring patients. Ideally, the developed sensor has been specific in detecting SARS-CoV-2 since the bioreceptor was created from the binding domain of the antibody CR3022. However, a mutation may affect the performance of the sensor, especially mutation on the RBD of S protein [40].

IV. CONCLUSIONS

The scFv CR3022 has the potential to be the bioreceptor for SARS-CoV-2 biosensor based on SPR. The optimum scFv was harvested from the periplasmic of E. coli and purified to remove the native proteins. Using samples of SARS-CoV-2 RBD, the purified periplasmic scFv has a maximum response at 8.02 RU, LOD at 8.34 ng/mL, linearity at 1.38 in range of 25-200 ng/mL, and determination coefficient at 92 percent. This developed sensor still needs an optimization in decreasing the limit of detection to work properly as a monitoring device for patients.

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