

RESEARCH PAPER

Affinity Peptide-based Electrochemical Biosensor for the Highly Sensitive Detection of Bovine Rotavirus

Chae Hwan Cho, Tae Jung Park, and Jong Pil Park

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Abstract Bovine diarrhea is a major concern in the global bovine industry because it can cause significant financial damage. Of the many potential infectious agents that can lead to bovine diarrhea, bovine rotavirus (BRV) is a particular problem due to its high transmissibility and infectivity. Therefore, it is important to prevent the proliferation of BRV using an early detection system. This study developed an affinity peptide-based electrochemical method for use as a rapid detection system for BRV. A BRV-specific peptide was identified via the phage display technique and chemically synthesized. The synthetic peptide was immobilized on a gold electrode through thiol-gold interactions. The performance of the BRV specific binding peptides was evaluated using square wave voltammetry. The developed detection system exhibited a low detection limit (5 copies/mL) and limit of quantitation (2.14×10^2 copies/mL), indicating that it is a promising sensor platform for the monitoring of BRV.

Keywords: bovine diarrhea, bovine rotavirus, phage display, affinity peptide, electrochemical sensor

1. Introduction

Bovine diarrhea not only poses a serious threat to public health but also generates severe economic losses within the global bovine industry due to its high mortality and

morbidity rates [1,2]. It can be caused by noninfectious factors such as environmental stress, management practices, and nutritional issues or by various infectious agents including bacteria such as *Salmonella* spp., *Clostridium perfringens*, and *Escherichia coli*, viruses such as bovine coronavirus (BCV), bovine rotavirus (BRV), and bovine viral diarrhea virus, and protozoa such as *Coccidia* and *Cryptosporidium* [3,4]. In particular, BRV is a major cause of neonatal calf diarrhea in calves aged 15-45 days [5,6], who can shed a high viral load through their feces, meaning that widespread infection is possible from even minimal environmental contamination [7]. Therefore, it is important to identify and manage infected groups via effective virus detection to prevent proliferation.

Various analytical methods have been developed for the detection of BRV, including electron microscopy, antigen-capturing enzyme-linked immunosorbent assays, restriction fragment length polymorphism, immuno-fluorescence, reverse transcription-polymerase chain reaction (RT-PCR), nested/multiplex RT-PCR, and dot-immunoblotting [8]. Although these approaches exhibit high sensitivity, they still require time-consuming processes and need to be carried out in laboratories by professionals because they require expensive reagents and sophisticated instruments.

Affinity peptide-based biosensors have been developed as an effective method for detecting the H5N1 influenza virus [9], human norovirus [10], and human immunodeficiency virus [11]. Peptides are more useful than antibodies as a recognition biomolecule due to their small size, stability under unfavorable conditions, cost-effectiveness, and ease of handling. Electrochemical biosensors have also been developed for detection purposes based on interactions between biomolecules, with advantages that include not needing reagents or labels, a non-invasive operating process, the possibility of on-site analysis, and the use of simple instruments [12].

Chae Hwan Cho, Jong Pil Park*
Department of Food Science and Technology, Chung-Ang University,
Anseong 17546, Korea
Tel: +82-31-670-4703; Fax: +82-31-675-3108
E-mail: jppark@cau.ac.kr

Tae Jung Park
Department of Chemistry, Research Institute of Chem-Bio Diagnostic
Technology, Chung-Ang University, Seoul 06974, Korea

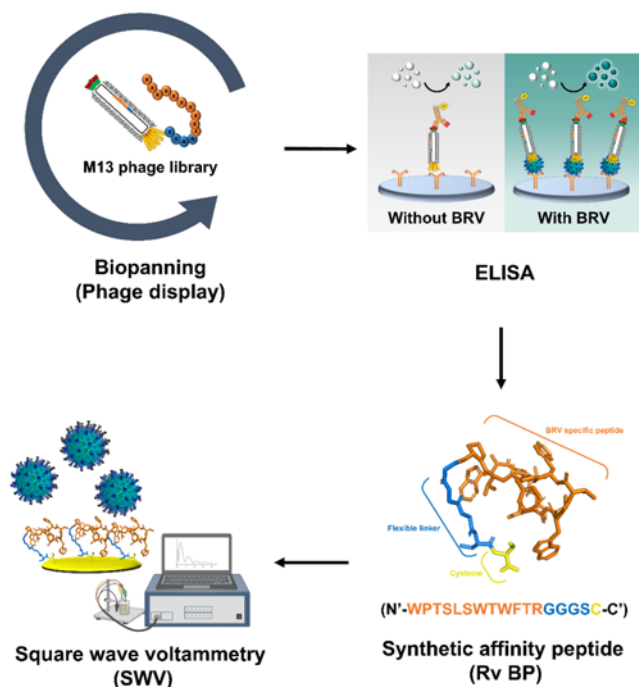


Fig. 1. Schematic illustration of the peptide-based electrochemical sensor for the detection of bovine rotavirus (BRV).

The objectives of the present study were to identify BRV affinity peptides using phage display and to develop an affinity peptide-based electrochemical biosensor using a synthesized BRV-specific peptide. To identify potential BRV-specific peptides, a random 12-mer peptide displayed phage library was introduced to a BRV-coated plate, and the amino acid sequences of the BRV-specific peptides were identified by sequencing. A BRV specific binding peptide with an amino acid sequence of WPTLSWTWFTR was chemically synthesized and coated onto a gold electrode. The performance of the affinity peptide-based electrochemical sensor was evaluated using square wave voltammetry (SWV), as shown in Fig. 1.

2. Materials and Methods

2.1. Chemicals

Phosphate-buffered saline (PBS) and all other chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA). Streptavidin-coated high-capacity plates (15500), an EZ-Link Sulfo-NHS-Biotinylation Kit, and a Pierce™ BCA Protein Assay Kit were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Deionized water at 18.2 MΩ cm was purified using a Purelab Elga® purification system (Elga, High Wycombe, UK). The PBS (pH 7.4) was used for peptide and virus sample preparation.

2.2. Screening of BRV-specific peptides using phage display technology

Novel affinity peptides were screened using the M13 peptide display library (New England Biolabs, Ipswich, MA, USA), which expresses random and linear 12-mer peptides. To create favorable conditions for analysis, a protein A coated plate was washed three times at 320 rpm with 200 μL of 0.1 M PBS (pH 7.4) for 30 sec. After removing the solution from the plate, 100 μL of 1 μg/mL BRV antibody diluted in 0.05% PBST (0.1 M PBS with 0.05% Tween-20) was added to the plate and stirred at 150 rpm for 60 min at 25°C. The plate was then rinsed three times with 200 μL of 0.05% PBST. Then, 100 μL of BRV (10^5 copies/mL) was added to the BRV-antibody-coated plate, which was placed in a 37°C incubator for 60 min. After incubation, the plate was rinsed three times at 320 rpm with 200 μL of 0.05% PBST. The M13 peptide display library (1×10^{11} pfu/mL) in 100 μL of 0.1 M PBS was added to the plate containing immobilized BRV and incubated for 60 min at 25°C. To remove unbound phage particles, the plate was rinsed 10 times with 0.1% PBST. The screened phage particles were then eluted using 100 μL of elution buffer (0.2 M glycine-HCl, pH 2.2) and rapidly neutralized with Tris-HCl buffer (pH 9.1; 15 μL) to prevent denaturation. To reduce the effect of non-specific interactions, the Tween-20 concentration in the washing buffer (PBST) was gradually increased (from 0.1 to 0.5%) depending on the progress of the biopanning. In addition, the M13 peptide phage library was pre-introduced to the BRV-antibody-coated plate without BRV before beginning rounds 2-4. Finally, we chose the candidate phages that bound specifically to the BRV based on their sequencing results.

2.3. General M13 methods and DNA sequencing analysis

E. coli strain ER2738 was used as a host for M13 phage infection and the titration, purification, DNA isolation, and amplification procedures were conducted according to the manufacturer's instructions. The DNA isolated from the phages was sequenced by Solgent (Daejeon, Korea) using the -96 pIII sequencing primer.

2.4. Biotinylation of the BRV antibody

The BRV antibody was biotinylated according to the manufacturer's instructions (Pierce Biotechnology, Rockford, IL, USA). Briefly, 100 μg/mL of the BRV antibody was mixed with 20 mM of biotin and the mixture was incubated at 4°C with ice overnight. After the incubation, the mixture of the BRV antibody and biotin was purified using a Zeba desalting column. The concentration of the biotinylated BRV antibody was determined using the BCA assay kit.

2.5. Characterization of the selected phage candidates using ELISA

The streptavidin-coated plate was pre-washed three times at 320 rpm with 200 μL of 0.1 M PBS for 30 sec. After removing the washing buffer, 100 μL of 1 $\mu\text{g}/\text{mL}$ biotinylated BRV antibody was added and the plate was incubated for 60 min at 37°C. The plate was then rinsed three times at 320 rpm with 200 μL of 0.05% PBST (0.1 M PBS with 0.05% Tween-20). The BRV antibody-coated plate was subsequently filled with 100 μL of 10^5 copies/mL BRV and incubated for 60 min at 37°C. The plate was then rinsed three times at 320 rpm with 200 μL of 0.05% PBST. To block the remaining surface, the blocking buffer (0.1 M NaHCO_3 , pH 8.6) and 5 mg/mL of bovine serum albumin were added to the plate and incubated for 60 min at 4°C. After removing the residual blocking solution, the plate was washed six times with 200 μL of 0.5% PBST (0.1 M PBS with 0.5% Tween-20). Following this, 100 μL of amplified phages ($\sim 10^{11}$ pfu/mL) was added to the plate, which was then incubated for 60 min at 25°C. The non-binding M13 phages were removed by washing six times at 320 rpm with 200 μL of 0.5% PBST. Subsequently, horseradish peroxidase (HRP)-conjugated anti-M13 monoclonal antibody (diluted 1:2,500 v/v in blocking solution) was added to the pre-treated plate and stirred at 150 rpm for 60 min at 25°C. After washing with 0.5% PBST six times, 100 μL of 2,2'-azino-di-3-ethylbenzthiazoline sulfonic acid (ABTS) was added to the plate and incubated for 15 min at 200 rpm. Finally, the plate was filled with 100 μL of stop solution (0.5 M H_2SO_4) and stirred at 200 rpm for 5 min. The ELISA signals were measured using an Epoch microplate spectrophotometer (BioTek, Winooski, VT, USA) at 450 nm.

2.6. Conjugation of a novel synthetic BRV binding peptides with a gold electrode

Based on the selected Rv-R3-15 phage particles with an amino acid sequence of WPTSLSWTWFTR, an Rv BP peptide with a cysteine residue and a flexible linker (–GGGS–) at the C-terminus was chemically synthesized by Pepton (> 90% purity; Daejeon, Korea).

To remove impurities, the gold electrode was chemically polished with 100% ethanol for 5 min and a piranha solution ($\text{H}_2\text{SO}_4:\text{H}_2\text{O}_2 = 7:3$, v/v) for 10 min. The gold electrode was then thoroughly rinsed with distilled water and dried with N_2 gas. After polishing, an electrochemical cell was assembled to fix the electrodes. The cysteine-incorporated synthetic peptides were then introduced to the gold electrode for specific immobilization via Au-S interactions. After incubation, the pre-treated gold electrode was rinsed five times with distilled water and 0.1 M PBS. Subsequently, freshly prepared BRV ($\sim 10^5$ copies/mL) was introduced to the peptide-immobilized gold electrode. Finally, the BRV-

coated electrode was washed five times with distilled water and 0.1 M PBS and dried with N_2 . The performance of the affinity peptide-based electrochemical sensor was evaluated using SWV.

2.7. SWV measurements

A typical three-electrode cell that used affinity peptide-coated gold as the working electrode, platinum wire as the counter electrode, and Ag/AgCl as the reference electrode was used for electrochemical analysis. SWV was conducted in 2 mL 1 M KNO_3 solution with 2.5 mM ferro/ferricyanide using a CHI 660E electrochemical workstation (CH Instruments Inc., Bee Cave, TX, USA) to monitor the binding behavior of the affinity peptide or BRV and recorded in the range of -0.3 to $+0.8$ V with a frequency and amplitude of 10 Hz and 50 mV, respectively.

The relative current change ($\Delta I\%$) for the SWV was calculated using the following relationship based on the change in the peak current obtained after the immobilization of the peptide and the BRV interaction:

$$\Delta I\% = (I_0 - I)/I_0 \times 100 \quad [13] \quad (1)$$

where I_0 and I denote the mean current after immobilizing the peptide and the mean current after binding to the BRV, respectively. The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using the following equations [14]:

$$\text{LOD} = 3 \times \sigma/S \quad (2)$$

$$\text{LOQ} = 10 \times \sigma/S \quad (3)$$

where σ refers to the standard deviation and S to the slope of the calibration line.

3. Results and Discussion

3.1. Identification of BRV-binding phage particles

Biopanning was carried out to identify novel BRV-specific affinity peptides using a 12-mer random peptide library. The phage library was introduced to the BRV, which was coated on the microplate by the BRV antibody. Despite the specific interactions of the antibody and antigen, some of the short peptide groups incorporated on the phage particles can be bound to the antibody through non-specific interactions such as hydrophobic and van der Waals forces. Therefore, we conducted a modified procedure to remove the antibody-affinity peptides. First, the phage library was reacted with the antibody-coated plate without BRV before introducing the phage library to the BRV-coated plate. After that, the pre-reacted phage library was added to the BRV-coated plate. The bound phage particles were eluted from the

Table 1. The enrichment of biopanning

Biopanning	Titer (pfu/mL)	Yield (%)
1st round		
Input	1.0×10^{11}	7.0×10^{-3}
Output	7.0×10^6	
2nd round		
Input	1.0×10^{11}	3×10^{-4}
Output	3×10^5	
3rd round		
Input	1.0×10^{11}	1.5×10^{-3}
Output	1.5×10^6	
4th round		
Input	1.0×10^{11}	6×10^{-3}
Output	6×10^6	

Table 2. Amino acid sequence and frequency of the identified phage particles

Name	Sequence	Frequency
Rv-R3-1	DHAQRYGAGHSG	5/80
Rv-R3-15	WPTSLSWTFWTR	13/80
Rv-R3-25	QMGFMTSPKHSV	5/80
Rv-R3-32	HFVKTPARWAWG	6/80
Rv-R3-34	LTPWIPGLVRDP	1/80

plates with an elution buffer (0.2 M glycine-HCl, pH 2.2).

Table 1 shows the enrichment for each round of biopanning with the 12-mer phage library. During the biopanning process, the yield of the phage particles decreased from round 1 to round 2 and increased from round 2 to round 4. This was due to the effect of removing the antibody-affinity peptide, which occurred during round 2 of the biopanning process. We obtained five phage clones from biopanning. Table 2 displays the amino acid sequence of peptides obtained via DNA sequencing.

From these observations, we confirmed the increased yield during biopanning and a common sequence with high probability, which is indicative of a well-controlled biopanning process [15]. Therefore, we selected five phage particle candidates and amplified them using the common procedure as previously mentioned. Their relative binding affinity for BRV was then evaluated using ELISA.

3.2. Selection and characterization of BRV binding phage particles using ELISA

The relative binding affinity of selected phage particles was compared using ELISA. Cross-reactivity can be observed between protein A and the HRP-M13 phage antibodies used to detect M13 phage particles in ELISA. Therefore, we used the biotinylated BRV antibody to coat the BRV on a streptavidin-coated plate. The biotinylated BRV antibody was first reacted with the streptavidin-coated plate, and BRV was then introduced to the BRV antibody, after which

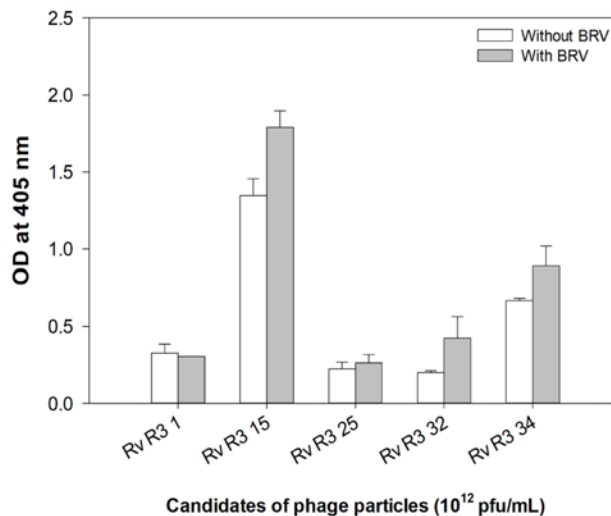


Fig. 2. Selection of bovine rotavirus (BRV)-specific phage particles. The biotinylated BRV antibody was first reacted with the streptavidin-coated plate, and BRV was then introduced to the BRV antibody. After incubation, phage particles were reacted with BRV. The HRP-M13 antibody was used to detect the bound phage particles and the change of absorbance was measured at 405 nm. All measurements were performed in triplicate and error bars represent standard deviations.

the phage particles were reacted with BRV. The HRP-M13 antibody was used to detect the bound phage particles. Finally, their absorbance as measured at 405 nm using ABTS. All phage candidates exhibited binding affinities for the BRV antibody itself (Fig. 2). We speculated that antibody could recognize the epitopes of antigen by interacting with the side-chain carbons and backbone atoms, through the interaction of functional paratopes. Therefore, our short peptides which displayed on the surface of phage particle has similar characteristics to the epitope and may act as recognition site for the BRV antibody. Under these circumstances, it was observed that affinity of Rv-R3-15 for BRV was higher than that of other phage candidates. The Rv-R3-15 phage particles exhibited much higher probability of conserved amino acid sequence during biopanning. The high affinity of Rv-R3-15 for BRV indicated a well-controlled biopanning and the successful screening of BRV affinity peptides. Therefore, we chose the Rv-R3-15 phage particles as a promising candidate for further analysis.

To investigate the effect of the phage concentration, we tested the binding affinity of the Rv-R3-15 phage particles at concentrations ranging from 10^{10} to 10^{12} pfu/mL (Fig. 3A). The binding affinity of the Rv-R3-15 phage particles increased in proportion with their concentration. This could be affected by the number of peptide molecules (normally three to five copies) that are present in a phage particle, thus the binding interaction of the peptides could increase by varying the density and concentration of the peptides.

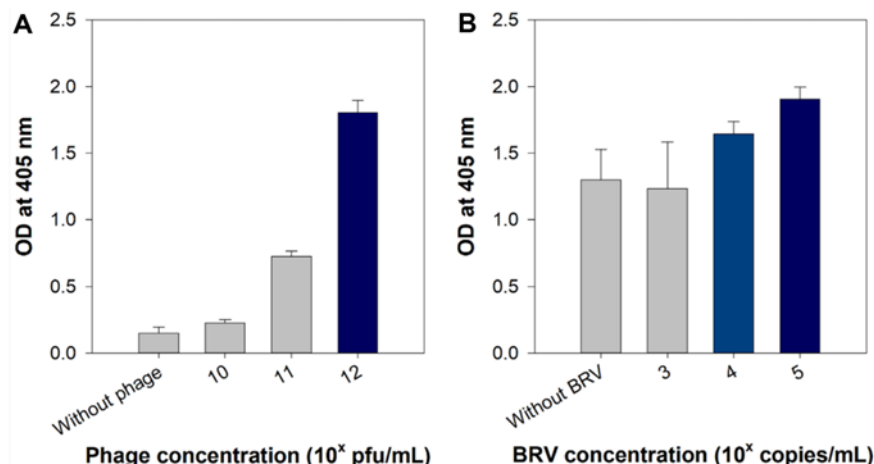


Fig. 3. Characterization of selected phage particles (Rv-R3-15). (A) Effect of the concentration of Rv-R3-15 phage particles on binding interactions. (B) Effect of the concentration of bovine rotavirus (BRV) on binding interactions. The relative binding affinities were measured by ELISA. All measurements were performed in triplicate and error bars represent standard deviations.

Different concentrations of BRV (10^3 - 10^5 copies/mL) were also immobilized on the plates to investigate the effect of the BRV concentration on the binding interactions (Fig. 3B). The binding affinity of the Rv-R3-15 phage particles increased in proportion to the increase in the concentration of BRV.

Thus, it was verified that the Rv-R3-15 phage particles had a good binding affinity for BRV. However, the sensitivity of the Rv-R3-15 phage particles was not sufficiently high for use in clinical detection. The affinity of peptides can be affected by chemical and physical interactions that occur with surface proteins on phage particles. Therefore, we separated the peptide from the phage particles to improve the sensitivity and affinity of the peptide receptor.

3.3. Synthesis of the BRV specific binding peptides

From the biopanning, we obtained a BRV-specific peptide with an amino acid sequence of WPTSLSWTFWTR. From this, we chemically synthesized the Rv BP peptide at a high purity (> 90%). The Rv BP peptide was synthesized with a cysteine residue incorporated at the C-terminal end to enable immobilization on the gold surface, which was employed as the working electrode for electrochemical analysis. The cysteine-incorporated synthetic peptide was strongly immobilized on the working electrode of the electrochemical sensor because the cysteine can bind to the gold surface through the thiol-gold interaction. In addition, a flexible linker (-GGGS-) was inserted between the sequence of the BRV-specific peptide and C-terminal cysteine. This linker plays a role in the interaction with BRV by increasing the flexibility of the gold-immobilized peptide [16]. Consequently, we obtained an Rv BP peptide with an amino acid sequence of WPTSLSWTFWTRGGGSC (MS found = 1,927.86, with a flexible linker [-GGGS-] and cysteine residue). The synthetic peptide was dissolved in

0.1 M PBS and placed in contact with the gold surface to obtain a peptide-coated gold electrode.

3.4. Optimization of the peptide-based electrochemical sensor

The change in the electrochemical behavior due to the interaction between the synthetic peptide and the gold surface was investigated using SWV measurements. SWV is widely used to investigate the binding interaction of target molecules with ligands because it provides insight into the binding behavior arising from the interactions between molecules. It is thus commonly employed in the development of highly effective electrochemical biosensors with high selectivity and sensitivity [17].

In the present study, the gold electrode was chemically treated to remove dust and impurities on its surface. The Rv BP peptide was then immobilized on the cleaned gold electrode at concentrations ranging from 0.7-70 $\mu\text{g/mL}$ to determine the optimal conditions for immobilization. The dynamic response was observed, and the Rv BP peptide was found to reach saturation at a concentration of 7 $\mu\text{g/mL}$ (Fig. 4A). The binding time for the peptide was also tested for a range of 30 to 300 min (Fig. 4B); the results showed that 120 min was the optimal binding time. Under the optimized conditions, the Rv BP peptide-coated gold electrode had a high reproducibility with a coefficient of variance of 7% (Fig. 4C), which suggests successful and uniform surface modification.

Fig. 4D demonstrates the feasibility of the sensor platform for BRV. The peak current varied in accordance with the modification of the gold electrode. After the immobilization of the Rv BP peptide, the peak current was lower than that of the bare gold electrode. In general, organic materials such as proteins and peptides can affect electrochemical

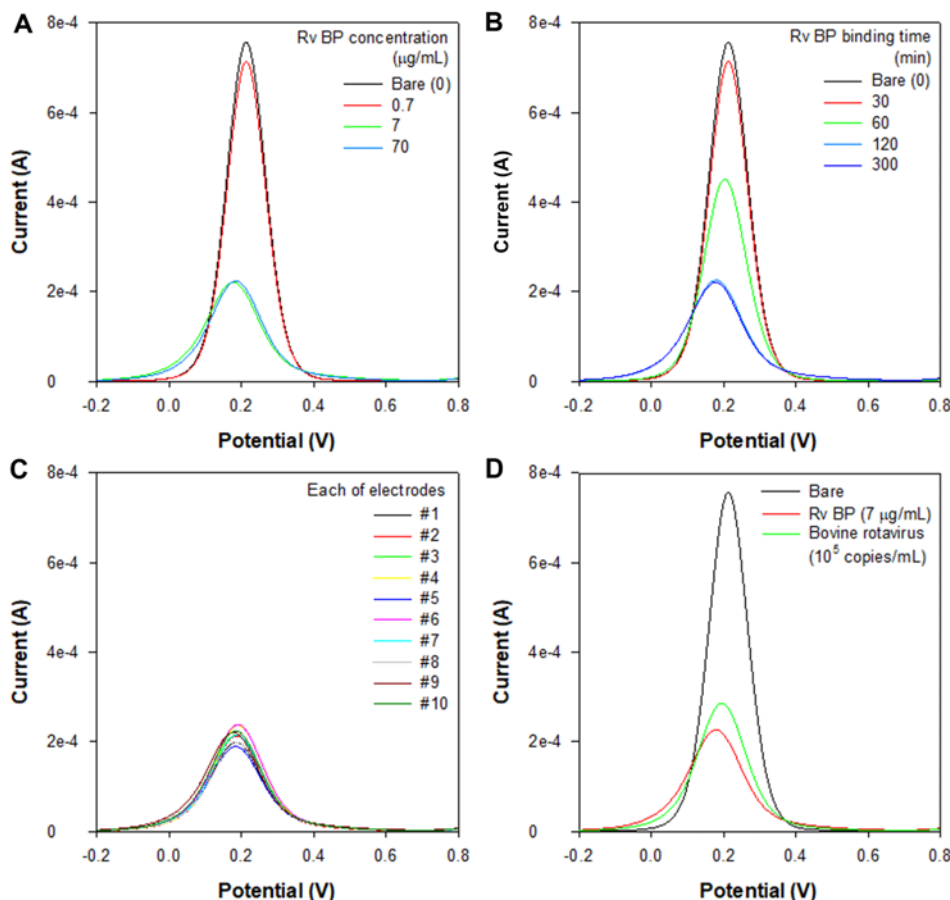


Fig. 4. Characterization of affinity peptide-based electrochemical sensors. (A) Optimization of Rv BP peptide concentration. (B) Optimization of reaction time between the peptide and gold surface. (C) Stabilization of current change after immobilization of Rv BP peptide on a gold electrode. (D) Feasibility of Rv BP peptide-based electrochemical sensor for bovine rotavirus detection.

behavior, such as the peak current, by acting as electrochemical insulators. In contrast, the peak current increased after the introduction of BRV to the peptide-immobilized gold surface. These observations were not in agreement with the expected theoretical variation, which could be attributed to protonation or the folding of the BRV structural proteins [18,19]. In particular, if the BRV structural proteins are positively protonated, the concentration of the redox probe ($\text{Fe}^{3+}/\text{Fe}^{4+}$) at the surface can decrease due to ionic interaction. In addition, the folding of BRV structural proteins could lead to higher electron accessibility and a greater rate of electron exchange with the redox probe ($\text{Fe}^{3+}/\text{Fe}^{4+}$) on the electrode surface.

3.5. Specificity of the peptide-based electrochemical sensor

Specificity is a vital attribute of a sensor. To evaluate the specificity of the Rv BP peptide, BCV was used as a control virus. The Rv BP peptide was coated on the gold electrode under the optimized conditions, and BRV or BCV at concentrations of 10^3 - 10^5 copies/mL were added to the Rv BP peptide-immobilized gold electrode. The relative

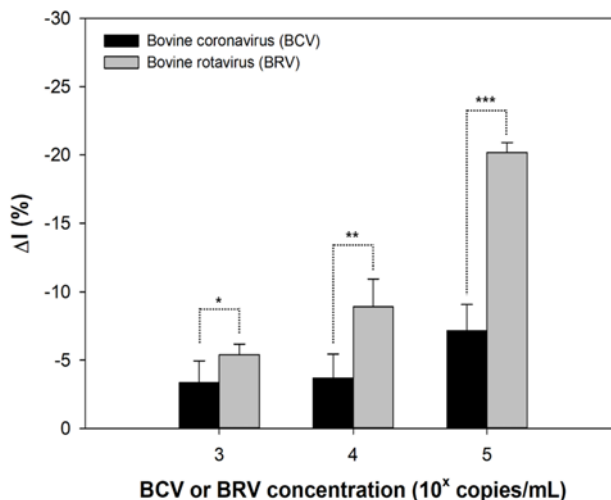


Fig. 5. Specificity of the Rv BP peptide compared to that of BCV. The square wave voltammetry measurements were performed to investigate the specificity with different bovine virus. Statistical significance between groups was determined by *t*-test, where **p* < 0.05 or ***p* < 0.001 or ****p* < 0.001. All measurements were performed in five replicate measurements, and error bars represent standard deviations.

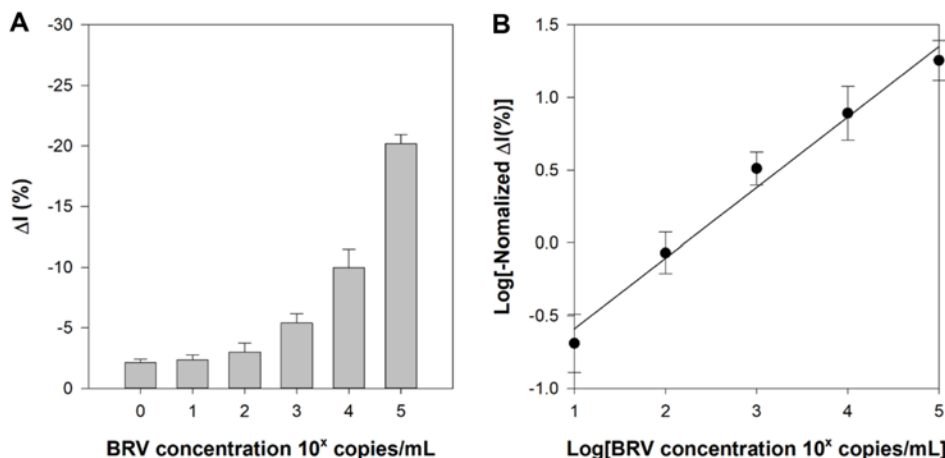


Fig. 6. Performance of the Rv BP peptide with square wave voltammetry measurement. (A) Effect of the bovine rotavirus (BRV) concentration on the binding interactions. (B) Construction of calibration graphs between the electrochemical signal intensities of Rv BP peptides-coated gold electrode and different concentrations of BRV. All measurements were performed in five replicate measurements, and error bars represent standard deviations.

change in the current (ΔI %) was calculated using the equation mentioned in Section 2.7 (equation (1)). As shown in Fig. 5, ΔI % gradually decreased with an increase in the concentration of BRV and showed statistically significant difference between BRV and BCV. However, it was observed that Rv BP peptide could recognize BCV. This might be due to non-specific binding interactions via hydrophobic or Van der Waals forces between Rv BP peptide and surface of BCV. These results indicated that there was a specific interaction between the Rv BP peptide and BRV and suggested that the interaction between the Rv BP peptide and BRV can affect the electrochemical behavior.

3.6. Analytical performance of the peptide-based electrochemical sensor

To evaluate the performance of the peptide-based electrochemical sensor, the Rv BP peptide-immobilized electrode was tested with BRV concentrations ranging from 0 to 10^5 copies/mL. It was found that ΔI % progressively decreased as the concentration of BRV increased (Fig. 6A). The LOD and LOQ were calculated from the slopes of the calibration curve obtained by plotting ΔI % vs. relative binding of the Rv BP peptide at various BRV concentrations. As shown in Fig. 6B, a strong linear correlation ranging from 10^1 to 10^5 copies/mL (saturation curve: $Y = 0.4851X + 1.0749$, $R^2 = 0.98$) was observed. Based on these observations, it was found that Rv BP peptide had a LOD (5 copies/mL) and LOQ (2.14×10^2 copies/mL) by using aforementioned equation (2) and (3).

The sensitivity of biosensor is considered as one of critical factors for validation of detection performance. For example, Wang et al. [6], reported that gold nanoparticle-based nanoPCR was found to have LOD (9.40×10^5

copies/mL) for BRV detection. From these facts, we concluded that our system exhibited significantly better detection limit. These suggest that the Rv BP peptide-coated gold electrode-based electrochemical biosensor exhibited the required selectivity and sensitivity for the detection of BRV, which illustrates its potential for use in applications for the analysis of BRV.

4. Conclusion

In summary, the BRV-specific peptide WPTSLSWTFWTR was identified using the phage display technique. This novel peptide was rationally designed and chemically synthesized for electrochemical applications. This synthetic peptide exhibited distinctive behavior on a gold electrode, allowing the effective detection of BRV. The BRV-specific peptide-based electrochemical sensor was evaluated using SWV and produced a good LOD and LOQ value. Interestingly, an increase in the peak current was observed after binding the BRV to the Rv BP peptide-coated gold electrode. Based on these observations, the Rv BP peptide-based electrochemical sensor successfully discriminated between BRV and BCV. In addition, the binding affinity of the Rv BP peptide to BRV gradually increased as the BRV concentration increased. Despite our affinity peptide-based electrochemical sensor had satisfactory outcomes, our sensor systems need to be further characterization for increasing sensor performances with more advanced sensitivity, stability, and selectivity. For examples, introduction of HRP or methylene blue as electron mediator enable to increase the electrochemical sensor performance [20,21]. In addition, some enzymes or chemicals have also used to improve the

electrochemical signal due to their characteristics, which can enhance the electrochemical signal intensities when activated by their corresponding substrates. Moreover, the use of gold nanoparticles allow for a more sensitive *E. coli* O157 detection [22]. In that studies, they suggested that gold nanoparticles-modified electrode system could enable more sensitive detection compared to those of planar gold electrode. Concerning these issues, we concluded that Rv BP peptide could be used as an alternative over conventional bioreceptors including antibodies and can be readily integrated into microfabricated detection systems combined with other nanomaterial-based electrode. Since phage display and electrochemical detection technique are widely used in diagnostics, this approach is straightforward and can be applied for developing more simple affinity peptide-based electrochemical sensor for new viral proteins or viruses.

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Ethical Statements

The authors declare no conflict of interest. Neither ethical approval nor informed consent was required for this study.

References

- Kim, M. W., H.-J. Park, C. Y. Park, J. H. Kim, C. H. Cho, L. Phan, J. P. Park, S. K. Kailasa, C.-H. Lee, and T. J. Park (2020) Fabrication of a paper strip for facile and rapid detection of bovine viral diarrhoea virus *via* signal enhancement by copper polyhedral nanoshells. *RSC Adv.* 10: 29759-29764.
- Beksisa, U., E. Melese, A. Helen, S. Tamirat, T. Markos, K. Temesgen, and A. Neima (2020) Antigen detection of bovine rotavirus infection in diarrheic crossbred dairy calves reared by Holeta Research Center, Oromiya Region Ethiopia. *Biomed. J. Sci. Tech. Res.* 30: 23242-23246.
- Cho, Y.-I. and K.-J. Yoon (2014) An overview of calf diarrhoea - infectious etiology, diagnosis, and intervention. *J. Vet. Sci.* 15: 1-17.
- Kim, W.-I., Y.-I. Cho, S.-J. Kang, T.-Y. Hur, Y.-H. Jung, and N.-S. Kim (2012) Simultaneous detection of major pathogens causing bovine diarrhoea by multiplex real-time PCR panel. *J. Vet. Clin.* 29: 377-383.
- Liu, X., N. Yan, H. Yue, Y. Wang, B. Zhang, and C. Tang (2021) Detection and molecular characteristics of bovine rotavirus A in dairy calves in China. *J. Vet. Sci.* 22: e69.
- Wang, M., Y. Yan, R. Wang, L. Wang, H. Zhou, Y. Li, L. Tang, Y. Xu, Y. Jiang, W. Cui, and X. Qiao (2019) Simultaneous detection of bovine rotavirus, bovine parvovirus, and bovine viral diarrhoea virus using a gold nanoparticle-assisted PCR assay with a dual-priming oligonucleotide system. *Front. Microbiol.* 10: 2884.
- Holland, R. E. (1990) Some infectious causes of diarrhoea in young farm animals. *Clin. Microbiol. Rev.* 3: 345-375.
- Dhama, K., R. S. Chauhan, M. Mahendran, and S. V. Malik (2009) Rotavirus diarrhoea in bovines and other domestic animals. *Vet. Res. Commun.* 33: 1-23.
- Kim, J. H., C. H. Cho, J. H. Shin, M. S. Hyun, E. Hwang, T. J. Park, and J. P. Park (2021) Biomimetic isolation of affinity peptides for electrochemical detection of influenza virus antigen. *Sens. Actuators B Chem.* 343: 130161.
- Hwang, H. J., M. Y. Ryu, C. Y. Park, J. Ahn, H. G. Park, C. Choi, S.-D. Ha, T. J. Park, and J. P. Park (2017) High sensitive and selective electrochemical biosensor: label-free detection of human norovirus using affinity peptide as molecular binder. *Biosens. Bioelectron.* 87: 164-170.
- Gerasimov, J. Y. and R. Y. Lai (2010) An electrochemical peptide-based biosensing platform for HIV detection. *Chem. Commun. (Camb.)* 46: 395-397.
- Choudhary, M. and K. Arora (2022) Electrochemical biosensors for early detection of cancer. pp. 123-151. In: R. Khan, A. Parihar, and S. K. Sanghi (eds.). *Biosensor Based Advanced Cancer Diagnostics: From Lab to Clinics*. Academic Press, London, UK.
- Cho, C. H., J. H. Kim, D.-K. Song, T. J. Park, and J. P. Park (2019) An affinity peptide-incorporated electrochemical biosensor for the detection of neutrophil gelatinase-associated lipocalin. *Biosens. Bioelectron.* 142: 111482.
- Pacheco, J. G., P. Rebelo, M. Freitas, H. P. A. Nouws, and C. Delerue-Matos (2018) Breast cancer biomarker (HER2-ECD) detection using a molecularly imprinted electrochemical sensor. *Sens. Actuators B Chem.* 273: 1008-1014.
- Cho, C. H., S.-Y. Son, J. K. Bang, Y. H. Jeon, and J. P. Park (2022) Biophysical and electrochemical approaches for studying molecular recognition of IL-33 binding peptides identified via phage display. *Anal. Chim. Acta.* 1197: 339522.
- Cho, C. H., J. H. Kim, J. Kim, J. W. Yun, T. J. Park, and J. P. Park (2021) Re-engineering of peptides with high binding affinity to develop an advanced electrochemical sensor for colon cancer diagnosis. *Anal. Chim. Acta.* 1146: 131-139.
- Chen, A. and B. Shah (2013) Electrochemical sensing and biosensing based on square wave voltammetry. *Anal. Methods.* 5: 2158-2173.
- Ben Hassine, A., N. Raouafi, and F. T. C. Moreira (2021) Novel electrochemical molecularly imprinted polymer-based biosensor for Tau protein detection. *Chemosensors. (Basel).* 9: 238.
- Bai, C., Z. Lu, H. Jiang, Z. Yang, X. Liu, H. Ding, H. Li, J. Dong, A. Huang, T. Fang, Y. Jiang, L. Zhu, X. Lou, S. Li, and N. Shao (2018) Aptamer selection and application in multivalent binding-based electrical impedance detection of inactivated H1N1 virus. *Biosens. Bioelectron.* 110: 162-167.
- Li, J., J. Wang, X. Guo, Q. Zheng, J. Peng, H. Tang, and S. Yao (2015) Carbon nanotubes labeled with aptamer and horseradish peroxidase as a probe for highly sensitive protein biosensing by postelectropolymerization of insoluble precipitates on electrodes. *Anal. Chem.* 87: 7610-7617.
- Puiu, M., A. Idili, D. Moscone, F. Ricci, and C. Bala (2014) A modular electrochemical peptide-based sensor for antibody detection. *Chem. Commun. (Camb.)* 50: 8962-8965.
- Lin, D., R. G. Pillai, W. E. Lee, and A. B. Jemere (2019) An impedimetric biosensor for *E. coli* O157:H7 based on the use of self-assembled gold nanoparticles and protein G. *Mikrochim. Acta.* 186: 169.

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