Phage Display-Derived Peptide for the Specific Binding of SARS-CoV-2

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ABSTRACT: Beginning from the end of 2019, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic swept all over the world and is still afflicting the whole global population. Given that the vaccine-manufacturing ability is limited and the virus can evolve quickly, vaccination alone may not be able to end the pandemic, thus developing fast and accurate diagnoses and effective therapeutics will always be unmet needs. Phage display peptide library has been used in screening antigen-specific peptides for the invention of novel mimic receptors/ligands. Here, we report that a 12-mer phage display peptide library has been screened against the SARS-CoV-2 receptor-binding domain (RBD), and five of the screened peptides show binding ability with the RBD protein by the enzyme-linked immune sorbent assay. The surface plasmon resonance assay further demonstrates that peptide no. 1 can specifically bind to SARS-CoV-2 RBD with a binding affinity constant ($K_d$) of 5.8 $\mu M$. Transmission electron microscopy coupled with a magnetic bead assay further confirms that the screened peptide can specifically bind the inactivated SARS-CoV-2 virus. This SARS-CoV-2-specific peptide holds great promise as a new bioreceptor/ligand for the rapid and accurate detection of SARS-CoV-2.

1. INTRODUCTION

The ongoing coronavirus disease 2019 (COVID-19) pandemic is still devastating and damaging public health globally. As of July of 2021, COVID-19 has infected over 180 million people and caused more than 4 million deaths all over the world. For infectious diseases, the best strategy to end the pandemic and return to a “new normal” is vaccination. Currently, there are several vaccines that are effective to protect humans from viral infection. Protection efficacy varies among different vaccines, especially to the recently emerged variants. However, the model has shown that vaccine alone is not enough to end the pandemic. Since everyone is susceptible to this virus, the large requirement for the vaccination of the entire world population has already challenged the vaccine production capacity of the manufacturers. Given that frequent evolution of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus may challenge the vaccine development in the foreseeable future, the virus will likely persist for a long time, like influenza and other pandemic viruses. Therefore, development of alternative diagnosis and therapeutics will always be unmet needs.

The gold standard diagnostics for COVID-19 are based on nucleic acid amplification by the reverse transcription polymerase chain reaction (RT-PCR). Compared to the fast diagnoses, the turnaround time for the RT-PCR testing results is much longer. Moreover, RT-PCR testing is time-consuming and needs expensive reagents and sophisticated instruments, and their applications are limited in developing countries and poor areas. Another strategy is antibody detection, but the antibody response is protracted. Thus, this strategy will not be feasible for early diagnosis when the antibodies have not been generated in the host or if the concentration is too low at the onset of viral infection. Additionally, the recently developed rapid SARS-CoV-2 antigen tests have generally low performance in terms of accuracy.

The phage display peptide library technology has been widely used to identify peptides that can specifically bind to antigen proteins. These peptides, due to their high binding affinity, can be used to detect the specific antigens. Compared with conventional antibody-based detection methods for antigen testing, which have usually been developed as enzyme-linked immunosorbent assays (ELISAs) or lateral flow testing kits, peptide-based detection is conceptually advantageous in terms of convenience and cost. Moreover, compared to proteins/antibodies, peptides...
can be generated faster and cheaper and can be stored at room temperature (RT). More importantly, peptides have fewer amino acids than proteins/antibodies, thus they are easier to be modified to improve affinity.\textsuperscript{18,19} Thus, peptides have been widely used to develop point-of-care diagnosis platforms.\textsuperscript{20,21} It is noteworthy that, even though the detection sensitivity of the direct detection using a peptide assay is lower than nucleic acid amplification, direct detection using a peptide assay is lower than nucleic acid amplification, will be useful to diagnose the patients, who already display symptoms and present a high viral load.\textsuperscript{22,23}

In the case of the SARS-CoV-2 virus, SARS-CoV-2 receptor-binding domain (RBD), located in the spike protein, is an important unit that the virus uses to bind and infect the host cells via the cellular receptor, angiotensin-converting enzyme 2 (ACE2).\textsuperscript{24–27} There are several SARS-CoV-2-specific peptides either derived from the ACE2 protein via computational design and/or affinity selection\textsuperscript{18,28,29} or screened from the mRNA display library.\textsuperscript{30} As the RBD is highly exposed outside the viral spike, it is a good target for screening against the phage display peptide library to obtain a potentially sensitive peptide for SARS-CoV-2 virus detection.

In this study, we screened a 12-mer phage display peptide library against the RBD of the SARS-CoV-2 spike protein. After three rounds of panning of the phage library, we obtained five SARS-CoV-2-specific phage-displayed peptides. We then synthesized these five peptides and characterized the binding affinity of the peptide to the RBD protein by a customized ELISA assay as well as surface plasmon resonance (SPR) assay and chose the one with the best binding ability for the inactivated SARS-CoV-2 virus-binding assay. The results indicate that the best peptide can specifically bind to the inactivated SARS-CoV-2 virus. We suggest that this peptide may potentially be utilized to develop a rapid, cost-effective antigen-based test (such as biosensors\textsuperscript{31,32}) to detect the SARS-CoV-2 virus.

2. MATERIALS AND METHODS

2.1. Materials. Recombinant SARS-CoV-2 spike RBD protein was purchased from Syd Labs Inc, Boston, MA (RBD protein, Cat # BP003052). Ph.D.—12 Phage Display Peptide Library and the screening kit were purchased from New England Biolabs, Ipswich, MA (Cat # E8110S). The M13 phage-specific antibody (Cat# PAI-26758) and horseradish peroxidase (HRP)-labeled goat antirabbit antibody (Cat # 31460) were purchased from Thermo Fisher Scientific Inc., Carlsbad, CA, while the rabbit anti-SARS-CoV-2 spike RBD antibody (Cat # 40592-T62) was purchased from Sino Biological Inc., China. The streptavidin (SA)-coated chips (Cat # BR100032) for the SPR measurement were purchased from Cytiva Life Science Inc., Marlborough, MA. All peptides were synthesized in Peptide 2.0 Inc., VA. The inactivated SARS-CoV-2 viruses (Isolate: USA-WA1/2020) were obtained from BEI Resources (https://www.beiresources.org/), while influenza A [A/Puerto Rico/8/1934 (H1N1)] hemagglutinin (HA) used as a negative control in the transmission electron microscopy (TEM) assay was purchased from the Native Antigen Company. The SARS envelope protein antibody used in the TEM assay was purchased from Novus Biologicals, LLC., Centennial, CO. N-Hydroxysuccinimide (NHS)-activated gold nanoparticles with a diameter of 40 nm were purchased from Cytodiagnostics Inc., Canada.

2.2. Phage-Displayed Peptide Library Screening. Recombinant SARS-CoV-2 spike RBD protein was used for Ph.D.—12 Phage Display Peptide Library (E8110S, New England Biolabs, MA) screening three times with increased stringency in the consequent rounds of screening. For instance, in the first round of screening, we coated the SARS-CoV-2 spike RBD protein at 100 μg and used a Tween-20 concentration of 0.1%. However, in the second round of screening, we decreased the RBD coating concentration to 50 μg while increased the detergent concentration to 0.25% to obtain the phages with a higher binding affinity to the SARS-CoV-2 spike RBD protein. In brief, in the first round, 2 mL of 100 μg/mL RBD protein in 0.1 M NaHCO₃, pH 8.6, was coated on a Nunc MaxiSorp flat-bottom plate (Cat # 44-2404-21, ThermoFisher) at 4 °C overnight. After removing the supernatant and blocking with 5% bovine serum albumin (BSA), the plate was washed six times with TBST (TBS +0.1% Tween-20). One hundred microliters of diluted phage library (with 1 × 10¹⁵ phage particles in 2 mL) was pipetted into each well and incubated at 37 °C for 1 h. The unbound phages were removed and washed 10 times with 0.1% TBST. The bound phages were then eluted with 100 μL per well of 0.2 M glycine–HCl (pH 2.2) with shaking at 110 rpm for 20 min, followed by neutralizing the buffer with 150 μL of 1 M Tris–HCl, pH 9.1.

2.3. Amplification of the Phage Sublibraries. The eluted phage library was amplified in 20 mL of ER2738 E. coli (OD600 at 0.05) with shaking vigorously at 37 °C for 4.5 h. The culture was then centrifuged at 12,000 g for 10 min at 4 °C, after which the supernatant was transferred into a new tube with the addition of one-sixth volume of 20% polyethylene glycol (PEG)/2.5 M NaCl and incubated at 4 °C overnight. The PEG-precipitated phages were then centrifuged at 12,000g for 15 min at 4 °C. The phage pellet was resuspended in 500 μL of TBS and that was the first SARS-CoV-2 RBD-specific sublibrary. This sublibrary was subjected to a phage titering and used for the next round of screening. The second and third rounds of screening were performed with the conditions as described in Table 1.

| Screening | Antigen con. (100 μg/mL) | Tween Con. (%) | Titer (PFU) |
|-----------|-------------------------|---------------|------------|
| First screening | 100 | 0.1 | 2 × 10⁶ |
| Second screening | 50 | 0.25 | 3.7 × 10⁴ |
| Third screening | 25 | 0.5 | 1.56 × 10⁵ |

2.4. Titering of the Phage Libraries. Titering of the phage sublibraries was performed as per manufacturing instructions. In brief, 10 μL of serially diluted (100-fold) phage sublibraries was mixed with 200 μL of midlog phase of E. coli ER2738 cells and the mixture was then added to 1 mL of prewarmed top agar and covered on a prewarmed LB/IPTG/ Xgal plate. After cooling the plates at 4 °C for 5 min, the plates were inverted and incubated at 37 °C overnight. The phage plaques were counted, and the titers were calculated according to the plaque numbers and the corresponding dilution.

2.5. Phage Peptide Sequencing. After each round of screening, 20 positive phages were randomly picked from each library by blue and white assay (IPTG/X-Gal) and amplified in
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Table 2. Synthesized Peptide Sequences

| hit | peptide sequence for first synthesis | peptide sequence for second synthesis |
|-----|-------------------------------------|--------------------------------------|
| 15  | Biotin-SCFDLNASWASCDDGGGS            | SCFDLNASWASCDDGGGS                   |
| 138 | Biotin-SCFDLNASWASCDDGGGS            | SCFDLNASWASCDDGGGS                   |
| 1   | Biotin-DVGLNWFITRGGGS               | DVMLNWFITRGGGS                      |
| 1   | Biotin-INQDARTMVPGGG                | INQDARTMVPGGG                       |
| 1   | Biotin-WAEKNHMYHMGMGGGS             | WAEKNHMYHMGMGGGS                    |
| 15  | Biotin-SC(CAM)FDFLNASWASCDDGGGS     | SC(CAM)FDFLNASWASCDDGGGS            |

5 mL of the culture. The individual phage genomic DNA was extracted, and the PCR was performed using the M13 primer that covers the DNA sequence encoding the whole length of the peptide. The PCR fragments were cloned into a T-A cloning vector, and DNA sequencing was performed at Eton Bioscience Inc, CA.

2.6. Phage ELISA. The phage sublibraries and the individual phages were subjected to phage ELISA to determine the binding affinity to the SARS-CoV-2 RBD protein. For this purpose, 10 μg of SARS-CoV-2 RBD protein in 0.1 M NaHCO₃, pH 8.6, was coated on a Nunc MaxiSorp 96-well plate at 4 °C overnight. After blocking with 5% skimmed milk, the plate was washed six times with 0.1% TBST, and the supernatants from the phage culture were applied to the plates and incubated at 37 °C for 1 h. After washing six times, the rabbit anti-M13 phage polyclonal antibody (1:1000 in TBST) was incubated in the plate for 1 h. The plate was washed six times to get rid of the nonspecific antibody and then coated with HRP-labeled goat antirabbit IgG for 30 min, followed by color development with the addition of the 3,3′,5,5′-tetramethylbenzidine (TMB) substrate. The OD values at 450 nm were recorded in a BioTeck Synergy 2 plate reader.

2.7. Peptide Synthesis. After phage ELISA, we chose five peptides that show the best performance for synthesis. Considering that the first peptide has two cysteines that may form intramolecular disulfide bonds, we also synthesized a peptide using the same sequence as peptide 1 but with a carbamidomethylation modification at the first cysteine (Table 2). The peptides synthesized at the first time were used for ELISA characterization. However, to immobilize the peptides on the SPR chips, we modified the N-terminal of the peptides with a biotin to bind to SA-occupied chips. The peptide syntheses were designated to Peptide 2.0 Inc., VA. Briefly, the peptides were synthesized using a solid-phase peptide synthesis method and Fmoc strategy. To maintain the consistency, we synthesized all peptides using a solid-phase peptide synthesis method and Fmoc strategy. We added 10 μL of the viral lysates into a vial containing lyophilized NHS-activated gold nanoparticles and incubated the vial at RT for 2 h. After that, 10 μL of the quencher solution was added into the mixture to stop the reaction. The vial was centrifuged for 30 min (speed: 900g). The supernatant containing unbound antibodies was discarded. Finally, the conjugate was resuspended in 100 μL of 1× PBS buffer and stored at 4 °C for later use.

2.8. Peptide ELISA. The synthesized peptides were subject to a customized ELISA assay. Briefly, the synthesized peptide was coated on a maleic anhydride-activated plate (Thermo-Fisher, Carlsbad, CA) with a concentration of 5 μg/well in NaHCO₃, pH 8.6 at 4 °C overnight. Superblock solution (Cat# 37580, ThermoFisher, Carlsbad, CA) was used to block the plate for 2 h at 4 °C. The plate was then washed with poly(butylene succinate-co-butylene terephthalate) (PBST) five times with shaking at 250 rpm for 5 min and incubated with 5 μg/well SARS CoV-2 RBD for 2 h at 4 °C. The unbound protein was washed off with PBST. The rabbit antiSARS-CoV-2 spike RBD antibody (Cat# 40592-T62, Sinobiological, China) was applied to the plate at a 1:5000 dilution, and the plate was incubated for 1 h at RT. After washing the plate five times with PBST, the HRP-labeled goat antirabbit antibody was incubated on the plate for 1 h at RT, followed by the addition of the TMB substrate to visualize the reaction as described earlier.

2.9. Surface Plasmon Resonance. The SPR assay was used for characterizing the binding affinity of the peptides with the SARS-CoV-2 RBD protein. N-terminal biotinylated peptides were synthesized at Peptide 2.0 Inc., VA. One microliter of peptide (20 μg/mL) was used to bind to the SA sensor chip channel, which consistently gives a signal at 400–600 nm. Then, 100 μL of 1000, 800, 600, 400, and 200 μg/mL of phosphate-buffered saline (PBS)-diluted SARS-CoV-2 RBD protein was run through a Biacore 3000 (Cytiva Life Science Inc., Marlborough, MA), and the binding curves were recorded to calculate the equilibrium constants for the dissociation (K₅ values) of the peptides with the SARS-CoV-2 RBD protein.

2.10. Conjugation of Gold Nanoparticles with SARS Envelope Protein Antibodies. The SARS envelope protein antibodies were at 0.5 mg/mL with the supplied protein resuspension buffer. We then mixed 90 μL of the protein/reaction buffer into one vial containing lyophilized NHS-activated gold nanoparticles and incubated the vial at RT for 2 h. After that, 10 μL of the quencher solution was added into the mixture to stop the reaction. The vial was centrifuged for 30 min (speed: 900g). The supernatant containing unbound antibodies was discarded. Finally, the conjugate was resuspended in 100 μL of 1× PBS buffer and stored at 4 °C for later use.

2.11. Conjugation of Peptides and Binding of the Viruses. Before conjugation, the SA-coated magnetic beads were washed three times with 1× PBS buffer. We added 10 μL of peptide 1 (2 mg/mL) into 10 μL of the Dynabead solution and incubated at RT for 30 min. After incubation, the beads were washed three times with 1× PBS buffer to remove any unbound peptides. We then added 10 μL of the viral lysate into the magnetic bead solution and incubated at RT for 20 min. The beads were washed three times again and resuspended in 10 μL of 1× PBS.

2.12. Binding of Viruses and Antibodies. We added 10 μL of the gold nanoparticle-protein antibody conjugate into the washed beads prepared in the previous step. The sample was incubated on a rotary mixer at RT for 30 min. The beads were washed three times with 1× PBS buffer to remove excess gold nanoparticle–protein antibody conjugates and resuspended in 10 μL of nuclease-free water.

2.13. TEM Characterization. Virus-captured magnetic beads were imaged by TEM (JEOL JEM-2010). Before imaging, we diluted the bead solution into distilled water and added 10 μL of that solution onto the TEM grid. The dried beads were then imaged with HV = 200.0 kV, direct Mag = 20 kx.
2.14. Magnetic Bead-Conjugated Peptide Sandwich Assay. Ten microliters of the SA-coated magnetic beads (10 mg/μL) were conjugated with 10 μL of biotinylated peptide 1 (2 mg/mL) at RT for 30 min and blocked with 5% BSA in PBS with 0.25% Tween-20 at 37 °C for 1 h. After being washed with 1× PBST (PBS with 0.25% Tween-20), 10 μL of heat-inactivated SARS-CoV-2 (1.6 × 10⁵ TCID₅₀ per mL or influenza virus (1.6 × 10⁵ CEID₅₀ per mL) was added and incubated at RT for 20 min. The unbound viruses were washed away by 1× PBST three times. Then, 10 μL of the Au-antibody was added and incubated with the bead-captured viruses for 30 min at RT. The unbound Au-antibody was washed away by 1× PBST, and the beads were heated at 75 °C for 10 min to release the peptides and protein. The resultant supernatants were collected into transparent tubes to observe the developed color (bound gold).

3. RESULTS AND DISCUSSION

3.1. Screening of Phage Library with the SARS-CoV-2 RBD Protein. To obtain SARS-CoV-2 RBD-specific peptides, we screened a 12-mer phage display library containing 2 × 10¹¹ individual phages with a diversity of 2 × 10⁹ random peptides (100 copies of phage for each peptide) against SARS-CoV-2 RBD for several rounds, with gradually increased stringency in the next round of screening to enhance the binding affinity of sublibraries and enrich the most SARS-CoV-2 RBD-specific peptides (Figure 1a). The first round of screening yielded 2 × 10⁵ phage plaques from each phage sublibrary were picked up and amplified in E. coli. The genomic DNAs were extracted for DNA sequencing, and the intact sequences are listed.
Figure 2. Binding affinity of the synthesized phage display-derived peptides. (a) Five synthesized peptides were coated to the immunoplate and subjected to a direct ELISA. Coated with peptides but without the addition of the RBD protein (BSA instead) served as a negative control to monitor background from the peptides. The other two negative controls are coated with PBS, without (shown as PBS) or with RBD (shown as RBD) after the blocking step, which are, respectively, used to monitor the systemic and nonspecific bound RBD background. (b,c) Biotinylated peptides 1 (b) and 6 (c) were immobilized on the SA-coated chip, and SARS-CoV-2 RBD was run to test the affinity of the peptides. (b,c) Binding curves of peptides 1 and 6 to different concentrations of SARS-CoV-2 RBD. (d) Calculation of the binding affinity constant ($K_d$) of peptides 1 and 6 to SARS-CoV-2 RBD. The binding data were plotted with a standard direct hyperbola method.
coated RBD protein concentration and increasing the detergent concentration), we obtained higher titers in the second and third panning (Table 1). We also performed an ELISA to characterize the three sublibraries to check if affinity had changed. The ELISA results showed that the libraries, after the second round of screening, have a much higher OD_{450nm} value than the first sublibrary. In contrast, the OD_{450nm} value of the third sublibrary is almost the same as the second one, indicating that the screening reached saturation (Figure 1b). The process of the phage display biopanning can enrich the high-affinity peptides after each panning; therefore, the third sublibrary should have the most high-affinity peptides enriched, compared with the second sublibrary. Indeed, after three rounds of panning, we found that some specific peptides were enriched after comparing the sequences of 20 peptides for each round of panning (Figure 1c). Therefore, we stopped the screening of the phage libraries at that point and picked hundreds of individual single-phage clones for further characterization.

During the phage display peptide library screening using a single target protein, the screening condition is maintained, while the library is subjected to 3–8 rounds of selections, at which point the high-affinity peptides are enriched via each round.33,34 However, we adopted a distinct strategy that combined decreasing coating antigen concentration and increasing detergent concentration in each next round, thus enhancing the enrichment of the high affinity of peptide-displayed phages and improving screening efficiency. Our results show that three rounds of screening are sufficient to enrich the highly specific peptides to the SARS-CoV-2 RBD protein, demonstrated by the DNA-sequencing hits. We found that only 5 sequences enriched out of 177 single-phage clones, and among them, one sequence has 138 hits (peptide 2).

3.2. Binding Affinity of the Phage Display-Derived Peptides with the SARS-CoV-2 RBD Protein. We selected and subjected 177 single colonies of the enriched phages to phage ELISA. Using 1.5 as the cutoff OD_{450nm} value, we identified 156 positive-phage plaque colonies for phage DNA sequencing. Based on the sequencing information, only five peptide sequences occurred within the 177 colonies (Table 2), so we choose all five positive sequences for peptide syntheses and further analyses.

To characterize whether the synthesized peptides can also bind with the SARS-CoV-2 RBD protein, we coated the immunoplate with the five selected peptides and performed a direct ELISA assay. The ELISA results show that all five peptides can efficiently bind with the RBD protein. As shown in Figure 2a, all five synthetic peptides show an OD_{450nm} value at ~1.0 with the presence of the RBD (orange). On the other hand, without RBD, the OD_{450nm} values remain below 0.1 for all the peptides and are comparable with the PBS buffer solution (green), demonstrating that all the five peptides can bind to the SARS-CoV-2 RBD protein.

We further quantified the binding affinity of the peptides with the SARS-CoV-2 RBD protein by an SPR assay. To this end, we first immobilized the SARS-CoV-2 RBD protein on the surface and then we tested the five peptides (without Biotinylation) by the SPR analysis, and only peptide 1 showed significant binding with SPR (Supporting Information, Figure S2). We reasoned that the linear peptides are too small to be detected by SPR, while peptide 1 may form polymers due to the double cysteines in the molecule. To test this hypothesis, we changed the detection strategy by immobilizing the peptide and running the SARS-CoV-2 RBD protein solution to detect binding. In addition, we synthesized another peptide with the same sequence as peptide 1 but with the first cysteine blocked by a carbamidomethylation modification. After immobilizing the SA-coated SPR chip on Biacore 3000, we ran through the SARS-CoV-2 RBD protein. The binding events were recorded, and the raw data were plotted to generate binding curves. Among the six peptides, two with essentially the same sequence but one with a CAM modification demonstrated good binding curves. As shown in Figure 2b,c, both peptides had a similar pattern binding with RBD, in which both the association and dissociation show sharp curves, indicating that the binding has fast on- and off-rates. Meanwhile, both peptides bound to SARS-CoV-2 RBD in a significant concentration-dependent manner. After we fit the data with a standard direct hyperbola method (shown in Figure 2d) and a double-reciprocal method (data not shown), both peptides yield very similar results for Max binding as well as K_d values for RBD binding. The K_d value of peptide 1 is 5.8 μM, while peptide 6 has an affinity of ~19 μM (Figure 2d).

The peptide exhibiting the most avid binding based on the screening of the phage display library has two cysteines. When it is synthesized for further characterization, it can form intramolecular disulfide bonds, by which it may create a cyclic ring structure that increases the conformational rigidity of the whole peptide structure. These cyclic peptides represent a large class of bioactive molecules,35 which usually have a higher binding affinity to the target proteins than their linear forms.36 In another scenario, this peptide may form intermolecular disulfides that result in peptide homodimerization or forms polymers, which usually will improve the binding ability to the target proteins by the potential conformational changes with higher binding affinity and/or increasing binding avidity.37,38 Indeed, once we blocked the first cysteine in peptide 1 (peptide 6), the affinity constant (K_d) dropped 3.5 folds from 5.8 to 19 μM. Our results imply that peptide 1 may form a cyclic conformation, which may change the binding epitope structure to a favored conformation. Alternatively, the polymerization of peptide 1 may help improve the binding affinity/avidity.

It is worth noting that the binding affinity (K_d) of peptide 1 to the SARS-CoV-2 RBD protein cannot completely reflect the ability of the peptide to detect SARS-CoV-2. In the case of SARS-CoV-2, the binding ability is determined not only by the affinity of the peptide with RBD but also by the avidity to SARS-CoV-2. However, the binding curve of this peptide with RBD shows rapidity with the curve going up sharply, implying that K_{on} is high, even the dissociation is also quick. The overall binding strength of the peptide may still be strong because binding ability is highly affected by the number of binding sites.39 Indeed, each sphere of the SARS-CoV-2 particle has ~72 spike proteins (24 ± 9 trimers),30 so it is possible that after the peptide dissociates from one of the spikes of the virion, the peptide may quickly bind to another spike protein on the surface of the virion. This scenario would result in generally high binding strength (high avidity) with the virion, and as discussed earlier, the highly flexible property of the short peptide makes the next quick binding possible.

3.3. Peptide 1 Can Specifically Bind to the SARS-CoV-2 Virus. As shown above, peptides 1 and 6 can efficiently bind to the SARS-CoV-2 RBD protein, but whether they can bind to the virion remains untested. To test the binding of the peptide to the whole virion, we used a magnetic bead assay and
observed the binding under TEM. To determine binding specificity, we used influenza virus as a control. Figure 3a summarizes the experimental procedure for the characterization of peptide binding with the heat-inactivated SARS-CoV-2 virus using the magnetic bead assay and TEM. (b–e) Biotinylated peptide 1 was immobilized on SA-coated magnetic beads to capture the virus. The gold nanoparticle-labeled SARS-CoV-2 antibody was used to detect the virus. The viruses and gold nanoparticles were visualized using TEM. The influenza virus served as a negative control. (b,c) Four individual magnetic beads with the addition of 0.67 and 0.067 mg/mL of the SARS-CoV-2 target to the surface, showing that gold nanoparticles (dashed red circle) have conjugated onto the magnetic beads. (d,e) Four individual magnetic beads with the addition of 0.67 and 0.067 mg/mL of influenza virus. The scale bar is 100 nm.

Figure 3. Peptide 1 can specifically bind to the SARS-CoV-2 virus. (a) Schematic of the experimental procedure for the characterization of peptide binding with the heat-inactivated SARS-CoV-2 virus using the magnetic bead assay and TEM. (b–e) Biotinylated peptide 1 was immobilized on SA-coated magnetic beads to capture the virus. The gold nanoparticle-labeled SARS-CoV-2 antibody was used to detect the virus. The viruses and gold nanoparticles were visualized using TEM. The influenza virus served as a negative control. (b,c) Four individual magnetic beads with the addition of 0.67 and 0.067 mg/mL of the SARS-CoV-2 target to the surface, showing that gold nanoparticles (dashed red circle) have conjugated onto the magnetic beads. (d,e) Four individual magnetic beads with the addition of 0.67 and 0.067 mg/mL of influenza virus. The scale bar is 100 nm.
antibodies to bind the viruses and released the virus by heating at 75 °C. When comparing color development initiated by the gold nanoparticles, the tube containing SARS-CoV-2 is clear, while the influenza virus tube remains dark (Supporting Information, Figure S4). The scale bar is 100 nm.

Magnetic beads have been used in the microfluidic system for virus detection, including SARS-CoV-2 detection.41–43 In those cases, the antigen-specific antibody was usually used to conjugate to the magnetic beads for antigen binding, and a fluorescence-labeled secondary antibody (binding to another epitope) was used to amplify the signal for detection. In this study, we utilized a peptide screened from the phage display peptide library as a bait to bind to the SARS-CoV-2 virus. Compared to using an antibody to detect viral antigens, short peptides are much cheaper, especially when the high-affinity antibody is not available. Moreover, if the small peptide can specifically bind to viral antigens, it is more flexible than antigen–antibody binding and much easier to fit in different detection platforms44–45 due to the relatively simple structure. In this study, we immobilized SA on the magnetic beads, and then, the peptides were efficiently conjugated to the beads via the SA–biotin interaction. Our TEM results show that the magnetic beads could efficiently catch the SARS-CoV-2 virus particle but not influenza virus, demonstrating high specificity to SARS-CoV-2. We further show that when we used a sandwich assay using this magnetic bead-conjugated peptide 1, the gold nanoparticle-labeled secondary antibody enriched when testing with the SARS-CoV-2 sample and the color was visible, while the influenza remained dark in color. These results demonstrated the potential of peptide 1 to be developed as a SARS-CoV-2 detection kit. It should be mentioned that there are several SARS-CoV-2 variants circulating in the world, some of which have become the dominant strains.46–47 Unfortunately, due to the lack of resources and accumulating variants, we have not tested the binding of our peptides with different variants. Moreover, we have not identified the binding epitopes of the peptides on the RBD motif. Nevertheless, if any of these peptides bind to the highly conserved sites of SARS-CoV-2, it should be able to be used to detect a wide spectrum of variants. We will explore the binding efficiency of different variants in our future work. We also note that, even though our peptides cannot distinguish live and dead viruses, a detection kit like this can be used to trace the transmission route in a disease outbreak. As a qualitative method, our peptides can report the overall viral load in the sample but are not designed to differentiate the infection cycles. Nevertheless, in the clinical settings, the therapeutic strategies to treat either primary or secondary infection are the same, once the COVID-19 testing is positive, and if the symptoms appear, the patients would be treated accordingly. Therefore, even if our method cannot differentiate primary infection from secondary infection, it is still clinically significant.

ASSOCIATED CONTENT

Supporting Information
The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c04873. Mass spectrometric characterization of peptides, binding curve of SARS-CoV-2 RBD to peptide 1, SARS-CoV-2-specific gold nanoparticle counts under TEM, and peptide-SARS-CoV-2 bead assay (PDF)

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