ORIGINAL RESEARCH PAPER

Design of fusion protein for efficient preparation of cyanovirin-n and rapid enrichment of pseudorabies virus

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Abstract

Objective Cyanovirin-N (CVN) is a cyanobacterial protein with potent neutralizing activity against enveloped virus. To achieve the economic and functional production of CVN, the CVN *N*-terminally fused with CL7(A mutant of the Colicin E7 Dnase) was utilized to improve the solubility and stability of CVN fusion protein (CL7-CVN). Additionally, to improve the detection limit of existing PRV diagnostic assays, CL7-CVN was used for Pseudorabies virus (PRV) enrichment from larger sample volumes.

Results CVN fused with CL7 was efficiently expressed at a level of $\sim$ 40% of the total soluble protein in *E. coli* by optimizing the induction conditions. Also, the stability of CVN fusion protein was enhanced, and 10 mg of CVN with a purity of $\sim$ 99% were obtained from 1 g of cells by one-step affinity purification with the digestion of HRV 3C protease. Moreover, both purified CVN and CL7-CVN could effectively inhibit the infection of PRV to PK15 cells. Considering the bioactivity of CL7-CVN, we explored a strategy for PRV enrichment from larger samples.

Conclusions CL7 effectively promoted the soluble expression of CVN fusion protein and improved its stability, which was meaningful for its purification and

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application. The design of CVN fusion protein provides an efficient approach for the economical and functional production of CVN and a new strategy for PRV enrichment.

Introduction

Globally, millions of human beings and animals are exposed to the enveloped viruses, threatening human health and the economy. Currently, effective antiviral drugs for the treatment of enveloped viruses are unavailable. Vaccines are used to inhibit the development of the epidemics, but the vaccines cannot eliminate the virus infection when the mutant strains appear frequently. Cyanovirin-n (CVN) initially discovered and isolated from *cyanobacteria* shows to be an effective antiviral protein for multitudinous enveloped viruses, such as Influenza A and B viruses, Herpes simplex virus type-1, and Human immunodeficiency virus (Smee et al. 2008; Buffa et al. 2009; Tiwari et al. 2009). CVN combines the highly glycosylated proteins of the enveloped virus with high affinity via two carbohydrate-binding domains, which prevents the virus from invading and adhering (Lusvarghi et al. 2016). Importantly, the antiviral activity of CVN is unaffected by the virus mutations that escape from immune system suppression. Therefore, its effective bioactivity and stable physicochemical properties make it possible for CVN to be a potential antiviral drug. Currently, CVN has been tried to be produced by eukaryotic expression system and prokaryotic expression system (Mori et al. 2002; Sexton et al. 2006). However, there are still some obstacles to the efficient preparation of CVN due to the formation of dimers, glycosylation modification, and downstream processing (Gao et al. 2010; Madeira et al. 2016). Even so, *E. coli* is still the preferred system for heterologous proteins production because of its simple culture conditions, no special instruments, convenient operation and short production cycle (Wurm et al. 2017; Kleiner-Grote et al. 2018).

The CL7/Im7 purification system is used for one-step purification of various biological molecules (Vassylyeva et al. 2017). When we purified the proteins with the purification system, we inadvertently found that CL7 promoted the expression of some specific proteins, and could even resist ~ 90 °C. Besides, CVN can remain in boiling water for 15 min. Here, CVN fused with CL7 is utilized to improve the soluble expression and purification of CVN (Vassylyeva et al. 2017). The results suggested CL7-CVN was highly and stably expressed with excellent stability in *E. coli*. Moreover, the high purity and activity CVN could be obtained by one-step affinity purification with Ni–NTA column. Furthermore, considering the biology of CVN fusion protein and the high affinity of CL7/Im7 (Kd $\sim 10^{-14}$–10^{-17} M) (Vassylyeva et al. 2017), we proposed a strategy for PRV enrichment based on the affinity of CL7-CVN to enveloped viruses and Im7 Beads to improve the virus detection rate.

Materials and methods

Strains, viruses and cells

The *E. coli* strains DH5α and Rosetta (DE3) were purchased from Sangon (Shanghai, China) and preserved by our laboratory. PRV (The UL21 gene of PRV was replaced by EGFP, ΔUL21/EGFP) and PK15 cells (Porcine kidney 15 cell line) were kindly provided by Huazhong Agricultural University, Wuhan, Hubei, China.

Protein expression and purification

According to the codon usage bias of *E. coli*, the nucleotide sequence of CVN (GenBank: KJ632500) was optimized and synthesized for protein expression in *E. coli*. The designed His-CVN and CL7-CVN were cloned into the pET28a plasmid. Then, the Rosetta(DE3) strains harboring these plasmids were grown at 37 °C in LB medium with 50 μg·ml-1 Kanamycin until the culture reached an OD600 = 0.6, then induced with 1 mM IPTG at 37 °C for 4 h. The cells were harvested and disrupted for testing protein expression by Tricine-SDS-PAGE (Fig. 1b).

To further improve the solubility of CVN fusion protein, the expression conditions for CVN fusion protein in *E. coli* Rosetta (DE3) were optimized by testing the supernatant and precipitates of the cells induced at various culture temperatures (18, 28, 37

°C), induction periods (12, 16 and 20 h), and IPTG concentrations (0.25, 0.5, 1.0 mM).

The typical procedures for the purifications of CVN and CL7-CVN were illustrated in (Fig. 2a). The supernatant of cell lysates CL7-CVN was treated at 80 °C for 30 min. Then, the purer supernatant of CL7-CVN was separated by centrifugation and loaded onto Ni–NTA column (GE Healthcare) for purification. The nonspecifically bound proteins were eluted with the buffer (10–30 mM imidazole, 20 mM Tris–HCl, 500 mM NaCl, pH 8.0) until the OD280 reached a base line. Subsequently, CL7-CVN was eluted with the buffer (300 mM imidazole, 20 mM Tris–HCl, 500 mM NaCl, pH 8.0). Briefly, CVN was released from the column with the digestion of HRV 3C protease, and undigested CL7-CVN, CL7 and HRV 3C protease stayed on the column.

Real-time PCR assay

Equal mole of purified proteins (0.25 μM of CL7, CVN, and CL7-CVN) were added to 100 μL of 100 TCID50/mL PRV and incubated at 37 °C for 1 h. Then, PK15 cells were incubated with PRV for 2 h. After changing the medium, culture was continued for 24 h. Afterwards, all of the RNA were carefully extracted from PK15 cells. Ultimately, cDNA was rapidly synthesized for real-time PCR analysis. The

assay was performed under the following conditions: 5-min activation of Taq DNA polymerase at 95 °C, followed by 40 cycles of 15 s at 95 °C, 45 s at 60 °C. The standard curve assay for mRNA was also performed.

50% tissue culture infective dose assays

The PK15 cells were placed in 96-well plates and incubated with 100 μL PRV particles at 37 °C for 1 h. After removing the culture medium, different concentrations (0.25, 0.5, 1, 2 μM) of CL7-CVN and CVN were added to incubate with cells for further 48 h. After the incubation period, the viruses were harvested and used for TCID50 assays.

Enzyme-linked immunosorbent assay (ELISA)

ELISA plates were evenly incubated with PRV particles in PBS at 37 °C for 2 h. The PBS containing 2% BSA was then employed to block at 37 °C for 1 h. Then, the different concentrations of CL7-CVN, CVN, and CL7 were added to ELISA plates and incubated at 37 °C for 1 h. The HRP substrates and Anti-His-tag HRP antibody were used to detect the conjugated CL7-CVN, CVN and CL7. Finally, the OD450 values were measured by Genios (Fig. 3d).
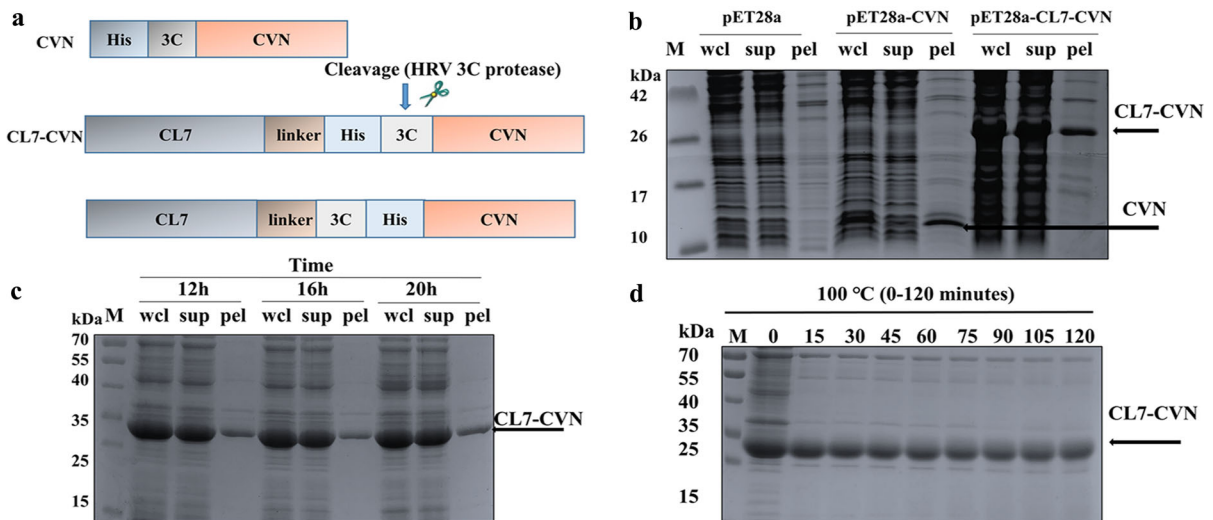

Fig. 1 The design and expression of CVN fusion protein. **a** Structure designs of CVN fusion protein for the expression of His-CVN and CL7-CVN. **b** The soluble expression of CL7-CVN in *E. coli*. **c** Optimization of CL7-CVN expression condition (Temperature, IPTG, Time).wcl, whole cell lysates; sup, supernatant of cell lysates; pel, pellet of cell lysates (pel). **d** Changes of supernatant protein in cell lysates treated at 100 °C for 0–120 min
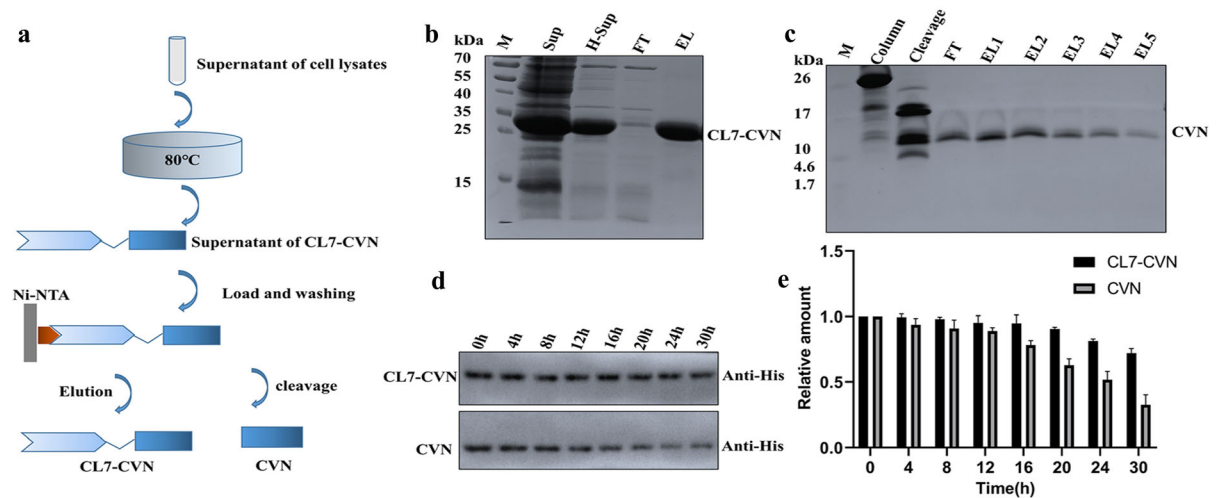
Fig. 2 Protein purification. **a** one-step affinity purification process for CVN and CL7-CVN. **b**, **c** One-step affinity purification of CL7-CVN and CVN. Sup, supernatant of cell lysates; H-Sup, supernatant of cell lysates heated; column, CL7-CVN on column; Cleavage, cleavage of CL7-CVN on the column; FT, flow through; EL, eluate; M, protein marker. **d** Western blot was used to detect the changes of CVN and CL7-CVN in serum. **e** The relative levels represent quantification of **d** using Image J software

Enrichment and detection of PRV

The highly purified Im7 was concentrated and immobilized for protein immobilization onto the Sulfo-Link (iodo-acetyl activated) 6B agarose beads as described in the study (Vassylyeva et al. 2017). The typical concentration of the immobilized Im7-unit was $\sim$ 15 mg/ml beads ($\sim$ 0.6 mM). Then, Im7 Beads were used for the enrichment of PRV.

The enrichment strategy was conveniently performed to capture and identify PRV (Fig. 4a). Briefly, Im7 Beads and CL7-CVN were added to the samples containing PRV particles and then incubated 30 min through gentle shaking at room temperature. Afterwards, the generated complexes (Im7 Beads-CL7-CVN-Virus) were rapidly separated by centrifugation. The viral DNA of the supernatant and pellet were extracted and measured by qPCR as described previously.

Results

Expression of CVN and CL7-CVN

After induction at 37 °C for 4 h, CL7-CVN was highly expressed in *E. coli*, and most of CL7-CVN were found in the lysate supernatant (Fig. 1b). In contrast, CVN without CL7 were basically in the lysate precipitation. The *N*-terminal CL7 performed as effective solubility-enhancing fusion tag and could be used for the soluble expression of CVN. To further improve the soluble expression of CVN, we preliminarily optimized the induction conditions of CL7-CVN expression (Temperature, IPTG concentration, Time). Briefly, the expression level was the higher ($\sim$ 40%) when IPTG of 0.25 mM was used to induce culture at 18 °C for 20 h (Fig. 1c).

Purification of CVN and CL7-CVN

The thermal stability of CL7-CVN was determined before protein purification. The most of proteins were denatured under heating at 100 °C, while the CVN fusion protein was intact, indicating that CL7-CVN had excellent thermal stability and could even exist at 100 °C for more than 2 h (Fig. 1d). Thus, we simplified the steps of CVN purification as described previously (Fig. 2a). In particular, the purity of CL7-CVN reached $\sim$ 80% after the supernatant of cell lysis is heated. Besides, the purity of CL7 -CVN reached $\sim$ 99% via additional one-step affinity purification with Ni–NTA (Fig. 2b). Moreover, 10 mg of CVN with a purity of up to 99% were obtained from 1 g of *E. coli* by one-step affinity purification with the digestion of HRV 3C protease (Fig. 2c). These demonstrated that
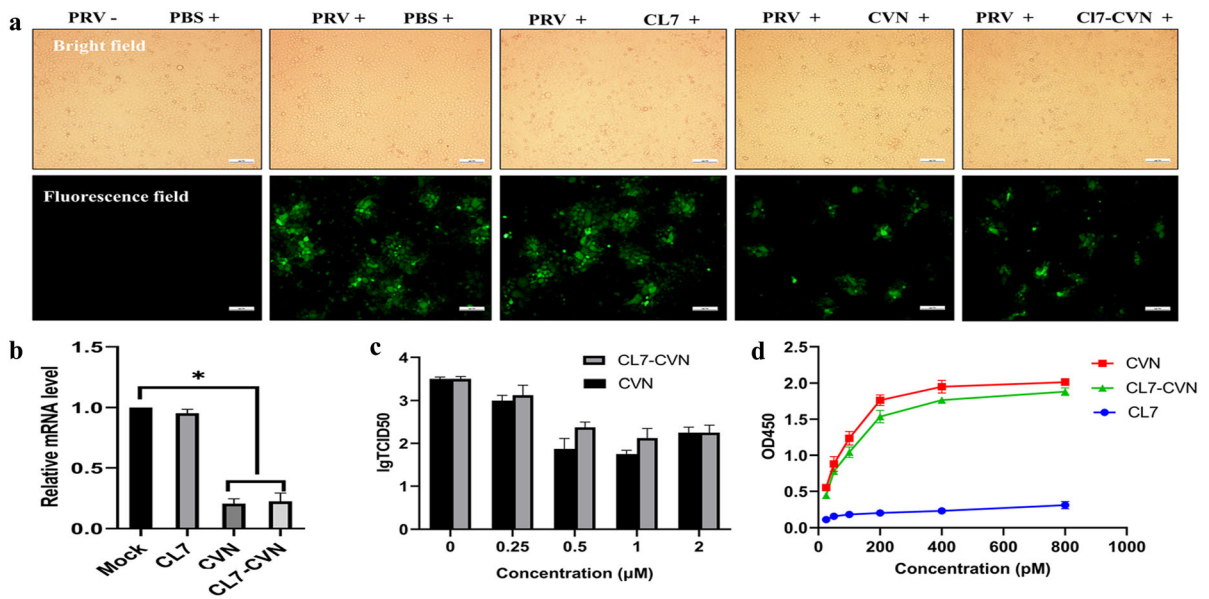
Fig. 3 Activity analysis of purified CVN and CL7-CVN. **a** Fluorescence microscopy images of PK15 cells infected by PRV treated with CL7-CVN, CVN, CL7 after culturing for 16 h. **b** The relative levels of mRNA of PRV in the infected PK15 cells treated with CL7-CVN, CVN, CL7 (*$p < 0.01$). **c** TCID50 assay of antiviral activity of CVN or CL7-CVN. **d** ELISA was performed to analyze the affinity of CVN and CL7-CVN to PRV
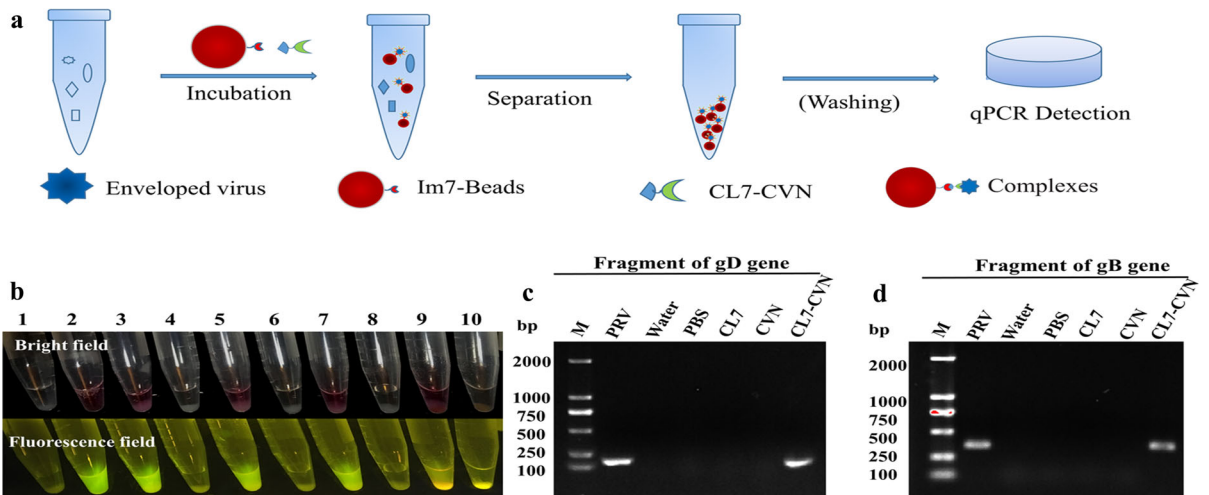

Fig. 4 The enrichment and detection of PRV. **a** Schematic of the strategy for the enrichment and detection of enveloped viruses using CL7-CVN with Im7 Beads. **b** The fluorescence intensity of PRV captured by Im7 Beads with PBS, CL7, CVN, and CL7-CVN. 1, Im7 Beads; 2, PRV sample; 3, PRV sample reacted with Im7 Beads and PBS; 4, PRV captured by Im7 Beads with PBS after washing; 5, PRV sample reacted with Im7 Beads and CL7; 6, PRV captured by Im7 Beads with CL7 after washing; 7, PRV sample reacted with Im7 Beads and CVN; 8, PRV captured by Im7 Beads with CVN after washing; 9, PRV sample reacted with Im7 Beads and CL7-CVN; 10, PRV captured by Im7 Beads with CL7-CVN after washing; **c, d** PCR analysis of PRV gene (206 bp fragment of gD gene, 331 bp fragment of gB gene) captured by Im7 Beads with PBS, CL7, CVN, and CL7-CVN; PRV, positive control; Water, negative control

CVN or CL7-CVN could be easily and economically purified using this system.

Stability analysis of CVN fusion protein

To further evaluate the stability of CL7-CVN, equal moles of CL7-CVN and CVN were respectively incubated with fresh chicken serum for 30 h at 37 °C. Western blot results demonstrated that the protein level decreased with the increase of incubation time (Fig. 2d). Moreover, CVN decreased by half when it was incubated for 24 h, yet CL7-CVN decreased by $\sim$ 28% when it was incubated for 30 h (Fig. 2e). CL7 significantly enhances the stability of CL7-CVN in serum, which is of great significance for its application.

Analysis of antiviral activity

After efficient production of proteins, the PRV(ΔUL21/EGFP) was considered as a virus model to determine antiviral activity of CVN. PK15 cells were infected with PRV treated with CL7-CVN, CVN, and CL7 at 0.25 μM. Comparing fluorescence intensity of PK15 cells, we recognized a significant decrease in multiplication of PRV treated with CVN or CL7-CVN after 16 h of culture (Fig. 3a). Besides, we measured the mRNA of PRV gD in PK15 cells via qPCR assay. The relative virus mRNA levels of CVN and CL7-CVN groups decreased by 79.59% and 77.64%, respectively, while CL7 did not significantly affect the mRNA level (Fig. 3b).

Besides, the TCID50 assay was used to assess the effect of CVN and CL7-CVN on PRV virulence. Compared to the negative control, PRV treated with CVN at 0.25, 0.5, 1 and 2 μM resulted in 0.5, 1.63, 1.75 and 1.25 lgTCID50 reduction, respectively. Meanwhile, CL7-CVN also resulted in 0.375, 1.125, 1.375 and 1.25 lgTCID50 reduction, respectively (Fig. 3c). The results showed that purified CL7-CVN and CVN inhibited PRV from infecting PK15 cells, indicating that the prepared CVN and CL7-CVN had eminent biological activity.

Furthermore, the ELISA was also implemented to characterize the affinity of CVN to PRV. CL7-CVN could bind to PRV as well as CVN, while CL7 could not recognize and combine PRV. Besides, with the decrease of CL7-CVN or CVN concentration, the OD$_{450}$ value decreased, suggesting its binding activity was dose-dependent (Fig. 3d).

Enrichment and detection of PRV

To improve the detection limit of existing PRV diagnostic assays, we explored a strategy for PRV enrichment utilizing Im7 beads with CL7-CVN. The process of virus isolation and enrichment was carried out as illustrated in (Fig. 4a). Im7 Beads treated with PRV sample and CL7-CVN had apparent fluorescence signal after washing. Conversely, the fluorescence signals of the control groups PBS, CL7, and CVN, weren't detected (Fig. 4b). Moreover, standard PCR assays for the enriched PRV were carried out. 206 bp fragments of the gD gene, 331 bp fragments of the gB gene, were respectively amplified from the PRV captured by Im7 Beads with CL7-CVN, whereas no fragments were observed when PRV samples were treated with Im7 Beads and PBS, CL7, CVN (Fig. 4c, d).

To further validate the reliability of our enrichment method, the amount of captured PRV was determined by qPCR. The PRV enrichment efficiency was calculated as the captured viral load divided by the initial viral load before the enrichment. A series of 100 μL PRV samples (10^6 viruses/μL) was prepared for virus enrichment utilizing CL7, CVN, and CL7-CVN at 0.25 μM, Im7 Beads at 0.5 mg/mL. The enrichment results showed that $\sim$ 90.78% of PRV were captured by Im7 Beads with CL7-CVN. Simultaneously, 20.93% of PRV were enriched by nonspecific physical adsorption by Im7 Beads with PBS (Fig. 5a). The efficiency of virus enrichment was calculated using different concentrations of CL7-CVN with Im7 Beads at 0.5 mg/mL. The results showed that the enrichment efficiency increased with the increase of CVN concentration in a certain range, and 97.32% of virus was captured at 0.5 μM of CL7-CVN (Fig. 5b).

Capability of PRV enrichment

To further assess the capability of our enrichment strategy, we tested the effect of sample size on the efficiency of virus enrichment. The 100 μL PRV samples (10^6 viruses/μL) were respectively diluted to different final volumes for PRV enrichment. When the viral sample was diluted to 1000 μL, the enrichment efficiency of virus had little change and reached
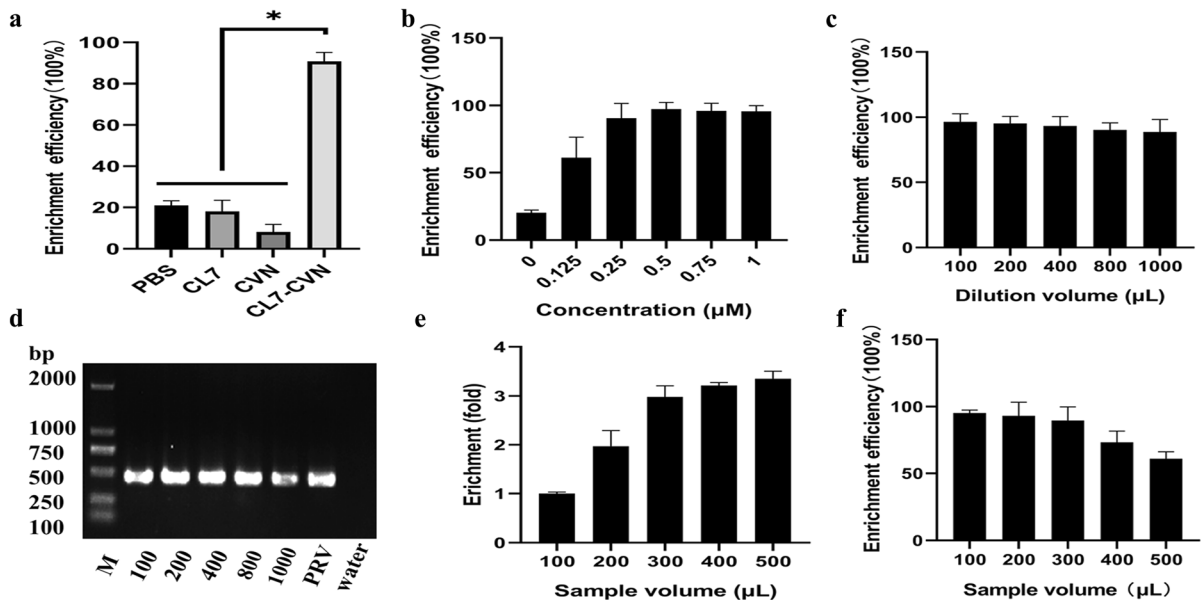
Fig. 5 Analysis of the enrichment capacity of PRV. **a** Different proteins were used to enrich virus samples (*$p < 0.01$). **b** Effect of CL7-CVN concentration on the enrichment efficiency. **c** Effect of sample size on the enrichment efficiency. **d** PCR analysis of 467 bp fragments of gK gene of PRV captured by Im7 Beads with CL7-CVN. **e** Viral load analysis of Im7 Beads. **f** Changes in enrichment efficiency of PRV in increasing sample volume (10^6 viruses/μL)

88.72% (Fig. 5c), and 467 bp fragments of the gK gene were also respectively amplified from the captured PRV (Fig. 5d). To investigate the viral load of Im7 Beads, 0.05 mg Im7 Beads immobilized with CL7-CVN was applied to concentrate PRV from the 100–500 μl samples (10^6 viruses/μL). When the virus sample volume increased from 100 to 300 μL (10^6 viruses/μL), the amount of the virus enriched increased to 3 times (Fig. 5e) and the enrichment efficiency of the virus also reached 89.64% (Fig. 5f).

Discussion

CVN has strong antiviral potential against a variety of enveloped viruses and may become a broad-spectrum antiviral drug (Smee et al. 2008; Buffa et al. 2009; Tiwari et al. 2009). However, the present production methods may be more or less defective. Here, we found the CL7-CVN we designed was highly soluble and functional expressed with excellent stability in the cytoplasm of *E. coli* (Vassylyeva et al. 2017). However, the mechanisms for increasing yield and improving solubility remain unclear. CL7 may affect the hydrophobicity and electrostatic repulsion of CVN

fusion protein, improving its solubility. Based on the thermotolerance and special structure design of CL7-CVN, we simplified the purification process of CVN, which greatly cut the production cost of this antiviral candidate. The expression and purification method described in this work allows for one-step purification of a wide range of traditionally antimicrobial peptide and heat-resistant protein.

Moreover, both purified CL7-CVN and CVN were biologically functional and had antiviral activities against PRV similar to others' enveloped viruses. Beside, we preliminarily performed the molecular docking of CVN and gD protein, indicating CVN and gD had the possibility of interaction but no details were described here. CVN contains two binding domains and inhibits HIV, influenza, Ebola, hepatitis C, and herpes viruses by binding to viral envelope proteins via the high mannose glycans (Smee et al. 2008; Buffa et al. 2009; Tiwari et al. 2009). These viruses all have a large number of highly glycosylated envelope proteins. The S-protein of SARS-CoV-2 has at least 66 glycosylation sites, which may make it possible for CVN to be a potential antiviral drug. In the future, we will further explore the mechanism of action between CVN and enveloped viruses.

Generally, efficiently concentrating virus from larger sample volumes can improve the detection signal. Based on the biological characteristics of CL7-CVN, we explored a strategy for PRV enrichment from larger samples (1 mL) employing the affinity of CL7-CVN to Im7 Beads and PRV. This method also had some non-specific virus enrichment ($\sim$ 20%), which might be caused by the non-specific physical adsorption of Beads or Im7 Beads. However, the nonspecifically separated virus did not impact the overall assay as the qPCR assay for the virus detection was specific because of the specificity of the primers. Furthermore, the non-specific enrichment can be reduced by multiple washing prior to further downstream assays.

Compared to using magnetic nanoparticles, this strategy employing Im7 Beads can significantly cut the enrichment time via rapid separation. Generally, the smaller magnetic nanoparticles need to be separated overnight because of low magneto phoretic mobility. While the Im7 Beads can be separated by centrifugation in 1 min. Additionally, the binding time between Im7 Beads, CL7-CVN and PRV can be optimized to decrease total enrichment time. Furthermore, the system utilized CL7-CVN for virus recognition, which did not depend on binding affinity of the antibody. Particularly, CVN could bind to the regions occluded by antibodies on glycoprotein. Compared to antibodies, CL7-CVN exhibits similar virus-binding ability with stable physicochemical property and more economical preparation methods (Wu et al. 2015).

In conclusion, we provided a simple, efficient and economical approach to produce the candidate antiviral drug CVN, and we explored a particular strategy to enrich whole virus for further downstream assays. Also, we wonder that the strategy may be extended to other targets and multiplexing. Future work will focus on rapid concentration and visual detection of viruses, as well as glycosylation detection of proteins.

Supplementary Information Supporting Information 1 Amino acid sequences for CL7-CVN: MGSKSNEPGKAT GEGKPVNNKWLNNAGKDLGSPVPDRIANKLRDKEFES FDDFRETFWEEVSKDPELSKQFSRNNNDRMKVGKAPKT RTQDVSGKRTSFELNHQKPIEQNGGVYDMDNISVVTPK RNIDIEGGGGGSGGGGSHHHHHHLEVLFQGPLGKFSQT CYNSAIQGSVLTSTCERTNGGYNTSSIDLNSVIENVDGSL KWQPSNFIETCRNTQLAGSSELAAECKTRAQQFVSTKIN LDDHMANMDGTLKYE

Supporting Information 2 SDS-PAGE analysis of purification of Im7: Figure S1. pET28a-Im7 was successfully

expressed and purified. The highly purified Im7 was then concentrated for protein immobilization onto the agarose beads to prepare Im7 Beads. The Im7 Beads were then used to capture viruses with CL7-CVN.wcl, whole cell lysates; sup, supernatant of cell lysates; pel, pellet of cell lysates; FT, flow through; EL, eluate.

Declarations

Conflict of interest The authors declare that they have no financial or commercial conflict of interest.

References

Buffa V, Stieh D, Mamhood N, Hu Q, Fletcher P, Shattock RJ (2009) Cyanovirin-n potently inhibits human immunodeficiency virus type 1 infection in cellular and cervical explant models. J Gen Virol 90:234–243

Gao X, Chen W, Guo C, Qian C, Liu G, Ge F, Huang Y, Kitazato K, Wang Y, Xiong S (2010) Soluble cytoplasmic expression, rapid purification, and characterization of cyanovirin-N as a His-SUMO fusion. Appl Microbiol Biotechnol 85:1051–1060

Kleiner-Grote GRM, Risse JM, Friehs K (2018) Secretion of recombinant proteins from E. coli. Eng Life Sci 18:532–550

Lusvarghi S, Lohith K, Morin-Leisk J, Ghirlando R, Hinshaw JE, Bewley CA (2016) Binding site geometry and subdomain valency control effects of neutralizing lectins on hiv-1 viral particles. Acs Infectious Dis 2:882–891

Madeira LM, Szeto TH, Ma KC, Drake PMW (2016) Rhizosecretion improves the production of cyanovirin-n in nicotiana tabacum through simplified downstream processing. Biotechnol J 11(7):910–919

Mori T, Barrientos LG, Han Z, Gronenborn AM, Turpin JA, Boyd MR (2002) Functional homologs of cyanovirin-N amenable to mass production in prokaryotic and eukaryotic hosts. Protein Expr Purif 26:42–49

Sexton A, Drake PM, Mahmood N, Harman SJ, Shattock RJ, Ma JK (2006) Transgenic plant production of cyanovirin-N, an HIV microbicide. FASEB J 20:356–358

Smee DF, Bailey KW, Wong MH, O"Keefe BR , Gustafson KR, Mishin VP, Gubareva LV (2008) Treatment of influenza a (h1n1) virus infections in mice and ferrets with cyanovirin-n. Antiviral Res 80(3):266–271

Tiwari V, Shukla SY, Shukla D (2009) A sugar binding protein cyanovirin-n blocks herpes simplex virus type-1 entry and cell fusion. Antiviral Res 84(1):67–75

Vassylyeva MN, Klyuyev S, Vassylyev AD, Wesson H, Zhang Z, Renfrow MB, Wang H, Higgins NP, Chow LT, Vassylyev DG (2017) Efficient, ultra-high-affinity chromatography in a one-step purification of complex proteins. Proc Natl Acad Sci USA 114(26):e5138–e5147

Wu M, Zhang ZL, Chen G, Wen CY, Wu LL, Hu J, Xiong CC, Chen JJ, Pang DW (2015) Rapid and quantitative detection of avian influenza a(h7n9) virions in complex matrices based on combined magnetic capture and quantum dot labeling. Small 11(39):5280–5288

Wurm DJ, Slouka C, Bosilj T, Herwig C, Spadiut O (2017) How to trigger periplasmic release in recombinant escherichia coli: a comparative analysis. Eng Life Sci 17(2):215–222