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Computational identification of self-inhibitory peptides from white spot syndrome virus envelope protein VP28

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\textbf{ABSTRACT}

Since effective chemotherapeutics or preventive measures are still unavailable, finding feasible approaches against white spot syndrome virus (WSSV) has always been the vital subject in shrimp farming field. Envelope proteins are the ideal targets for antiviral strategies development due to their indispensable roles in virus entry, and inhibitory peptides targeting them have been proved to be promising in blocking virus infection. In this study, the Wimley-White interfacial hydrophobicity scale (WWIHS) in combination with known structural data was applied to identify potential inhibitory peptides that targeted the envelope protein VP28 of WSSV. Results showed that two potential inhibitory peptides were identified, one of which exhibited not only obvious antiviral activity, but also broad-spectrum antimicrobial activity. The inhibitory peptide identified here can serve as a lead compound for anti-WSSV strategies development.

\section{1. Introduction}

As an important protein source for human consumption, shrimp cultivation has become a major economic and food production sector in many countries and regions (Lakshmi et al., 2013). However, this business has long been bewildered by white spot syndrome virus (WSSV), one of the most serious viral pathogens for shrimp, once resulting in enormous economic losses world-wide (Ramos-Carreño et al., 2014; Sivakumar et al., 2016; Tandel et al., 2017). Unfortunately, no effective chemotherapeutics or preventive measures are available now (Sivakumar et al., 2016).

For the purpose of finding feasible approaches against this virus, more and more studies have focused on the entry process, especially on the recognition and attachment of the virus to its host cell surface (Huang et al., 2013; Huang et al., 2014; Zhang et al., 2014; Huang et al., 2015). Since entry into the cell is the first step for the initiation, dissemination, and maintenance of virus infection, blocking this process will have a significant inhibitory effect. Considering the vital roles in the initial steps of the viral infectious cycle such as recognition and attachment, envelope proteins have become the ideal targets for antiviral strategies development (Yi et al., 2016; Dinesh et al., 2017; Priya et al., 2018; de Wispelaere et al., 2018), among which, design of antiviral peptides (AVPs) has lately regained interest following pioneering advancements in technology (Chew et al., 2017). By using this approach, peptides derived from several viruses have been designed and exhibited significant self-inhibitory activity (Ho et al., 2006; Costin et al., 2010; Schmidt et al., 2010; Xu et al., 2012; Koehler et al., 2013; Spence et al., 2014).

As a large enveloped virus, WSSV is currently known to have about 30 envelope proteins (Chang et al., 2010), including four major proteins (VP19, VP24, VP26 and VP28) (Zhou et al., 2009). There is increasing evidence that WSSV envelope proteins play very important roles in virus-host interactions by forming protein complexes (Huang et al., 2014; Zhang et al., 2014; Chang et al., 2010; Zhou et al., 2009; Lee and Chen, 2017). As the most abundant surface protein, the major protein VP28 can interact with many other proteins (Zhou et al., 2009; Yi et al., 2004; Xu et al., 2009; Li et al., 2012), and participate in formation of most known protein complexes (Zhou et al., 2009; Li et al., 2011), acting as a vital virulence factor in the initial phases of WSSV infection in shrimp (Sivakumar et al., 2016), therefore has been considered as an ideal target for WSSV controlling (Nguyen et al., 2014; Nilsen et al., 2017; Taengchaiyaphum et al., 2017; Taen-claiyaphum et al., 2017; Taju et al., 2018). Inhibition of VP28 may be a novel way to deal with infection by blocking its interaction in the endocytic pathway (Sivakumar et al., 2016; Zhan et al., 2018).

Hence, in this work envelope protein VP28 was targeted to design self-inhibitory peptides, which can be used as leads for anti-WSSV strategies development.

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2. Materials and methods

2.1. Virus and shrimp

The WSSV strain CN01 used in this work was maintained at the Yellow Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences. The shrimps with a body length of 7.0 ± 0.5 cm and a body weight of 4.5 ± 0.5 g were purchased from the shrimp farm and cultivated in air-pumped circulating sea water at 25 °C for a week before the experiments. The shrimps were sampled to check for potential WSSV infection by PCR (primers F:actactaacttcagcctatctag; R:taatgcgggtgtaatgttcttacga).

2.2. Computational identification of potential inhibitory peptides

The amino acid sequence of VP28 (GenBank:YP_009220614) was analyzed by the program Membrane Protein Explorer (MPEx version 3.2) (Snider et al., 2009), and the interface scale was used to identify regions with a positive Wimley-White interfacial hydrophobicity score (WWIHS) for peptide generation.

The secondary structure of the candidate peptide was evaluated by server SABLE (http://sable.cchmc.org/), which possessed a mean prediction accuracy over 80% (Adamczak et al., 2005). HeliQuest server was used to calculate the cationicity, hydrophobicity (H) and hydrophobic moment (μH) of the potential α-helical segment, whose helical wheel plot was also drawn via this server (http://heliquest.ipmc.cnrs.fr/) (Gautier et al., 2008).

2.3. Docking studies

Using 3D structure of VP28 [PDB:2ed6] as a target for the peptide 3D structure, peptide-protein docking was done by HDOCK server (http://hdock.phys.hust.edu.cn/), which is based on a hybrid algorithm of template-based modeling and ab initio free docking (Yan et al., 2017). The peptide 3D structure was modeled using the PEP-FOLD program, and the best 3D model for the peptide was selected for docking (http://bioserv.rpbs.univ-paris-diderot.fr/services/PEP-FOLD3/) (Shen et al., 2014). The docking model with the lowest docking energy score and the highest ligand root-mean-square deviation (RMSD) was selected to analyze their interaction. Docking results were visualized using Swiss-PdbViewer program (Johansson et al., 2012).

2.4. Synthesis of the peptide

Peptides were synthesized by a solid-phase conventional N-a-9-flurenylmethoxycarbonyl chemistry and purified by reverse-phase high performance liquid chromatography to greater than 90% purity (Synpeptide Co Ltd, Nanjing, China). Lyophilized peptides were dissolved by resuspending all peptides in 50% dimethyl sulfoxide (DMSO) (Aldrich, Shanghai, China) to a concentration of 100 μg/ml. The control peptide was generated by randomly scrambling the positions of amino acids in the experimental peptide.

2.5. Antiviral assay of the peptide

For WSSV challenge experiment, virus particles were centrifuged from the tissue homogenate of the infected shrimps, and proved by PCR using a pair of primers (F:actactaacttcagcctatctag; R:taatgcgggtgtaatgttcttacga). The extracted WSSV particles were suspended in sterile phosphate-buffered saline (PBS). WSSV challenge was done by injecting the shrimp with 20 μl WSSV solution containing about 10^4 copies of virus particles. In order to test the antiviral activity of the peptide, three treatments were designed. In treatment one, each shrimp in the experimental group was injected with 20 μl designed peptide two days before WSSV challenge; in treatment two, each shrimp in the experimental group was injected with 20 μl designed peptide solution two days after WSSV challenge; in treatment three, each shrimp in the experimental group was injected with 20 μl WSSV solution and 20 μl designed peptide solution simultaneously. In the control group of each treatment, the shrimps were injected with the same volume of virus as well as the control peptide at the same time of challenge.

![Diagram](http://example.com/diagram.png)
WSSV challenge of the experimental shrimps. There were 15 shrimps in each group. All shrimps were cultured in air-pumped circulating sea water with a temperature of 25 ± 1 °C. The assay was carried out in triplicate.

2.6. Antimicrobial activity analysis

Antimicrobial activity of the peptide was detected by standard disc diffusion method. To prepare the inoculum for the experiment, the test strains *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Shewanella baltica*, *Vibrio diabolicus* and *Saccharomyces cerevisiae* were inoculated into 5 ml nutrient broth by aseptic technique and incubated for 8–12 h at 37 °C, respectively, and then 30 μl culture of each strain was spread evenly on the surface of the agar media. After the liquid was absorbed, a total of 10 μl peptide solution (100 μg/ml) was dropped on the surface of each plate with a micropipette, 10 μl DMSO solution was also added as the control. The inoculated plates were incubated at 37 °C for 24 h. Zones of inhibition were applied for all plates to value the antimicrobial effects of the peptide. The assay was carried out in triplicate.

2.7. Statistical analysis

Statistical analyses were performed using Microsoft Office Excel 2010. P values of < 0.05 were considered significant.

Fig. 3. Docking of monomer of VP28 with the peptide. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article). Docking analysis was performed by HDOCK server, and the result was visualized using Swiss-PdbViewer program. a. H-bonds mainly formed between the C-terminal of the peptide and VP28, the green lines indicated H-bonds; b. Electrostatic potential of VP 28 and the peptide, the red indicated that of VP28, the blue at the top right indicated that of the peptide; c Docking complex in ribbon form, the helix at the top right was the peptide with hydrophobic residues marked red, the hydrophobic residues in β-strands of VP28 were marked blue.
3. Results

3.1. Identification of WSSV inhibitory peptides

Membrane Protein Explorer analysis showed that there were two regions with significant WWIHS values in the amino acid sequence of VP28, located at the position 11–29 of N-terminus (SAILAITAVIYFIFVFRY) and 170–199 of C-terminus (FVCGTTFGAPIAATAGGNLFDMYVHTVYS), respectively [Fig. 1], which were believed to preferentially interact with the hydrophobic surface within the envelope proteins and therefore had the potential to be developed into inhibitors. Since the former had a slightly higher score and less amino acids than the latter, it was chosen for further activity analysis.

3.2. In silico secondary structure analysis of the synthesized peptide

Bioinformatics analysis showed that this candidate peptide could form an α-helical and amphipathic structure, which could be seen from the helical wheel plot (Fig. 2); besides, it contained a positive charge, exhibiting typical features of antimicrobial peptides.

3.3. Docking analysis

Docking results showed the peptide could interact with the wall of the β-barrel from the monomer of VP28 by hydrogen bonds, electrostatic interaction and hydrophobic interaction [Fig. 3]. Since the protruding N-terminal region of VP28 may anchor on the viral envelope membrane, making the core β-barrel protrude outside the envelope, binding of the peptide may interrupt the formation of VP28 trimers in the viral envelope, or the interaction with other envelope proteins, ultimately preventing viral entry into the cytoplasm by fusion of their lipid envelope with the host cell membrane.

3.4. WSSV inhibitory activity

Antiviral assay showed the peptide couldn’t provide any protection on shrimps if applied two days before or after WSSV challenge (p > 0.05), but if injected together with virus particles, it exhibited obvious protective effect, especially within 24 h after WSSV challenge (p < 0.05); about 4 days later, all the shrimps in the control groups died, whereas 10 days later there still were shrimps alive in the test groups (Fig. 4).

3.5. Antimicrobial activity of the peptide

The antimicrobial activity of the peptide was presented in Table 1. At the concentration of 100 μg/ml, the peptide was found to be active against all the strains, showing broad-spectrum antimicrobial activity; since the peptide had high hydrophobicity, it was dissolved with DMSO, which didn’t show any antimicrobial activity in this study, so it didn’t influence the activity analysis of the peptide.

4. Discussion

The success of developing peptide inhibitors from HIV-1 gp41 into clinical application has triggered interests for the design of drugs targeting viral envelope proteins (Xu et al., 2012). Peptides derived from envelope proteins are supposed to be able to mimic the modes of binding of its original domain to its specific partner protein, thus, they may serve as potential inhibitors to disrupt the protein–protein interactions in membrane fusion mediated by the envelope proteins (Xu et al., 2012). Many peptides have exhibited good performance against the corresponding viruses, including dengue virus (Costin et al., 2010; Schmidt et al., 2010), SARS coronavirus (I ho et al., 2006), and most notably, HIV-1 (Yi et al., 2016), and the main feature of them is the high hydrophobicity, some computational tools based on this feature have been proved to be useful in identifying potential inhibitory peptides (Xu et al., 2012; Koehler et al., 2013; Spence et al., 2014).

There are mainly three types of envelope proteins: Class I are mainly α-helical structures, Class II are mainly β-sheet organization, Class III are generally a mixture of α-helix and β-sheet (Xu et al., 2012). Crystal structure analysis showed that VP28 should belong to Class III (Tang et al., 2007; Sun et al., 2016). Belonging to the same class, seven hydrophobic regions were identified from HSV-1 gB, whereas only two from VP28. The number of inhibitory peptides differs among various envelope proteins, which should be related to their different size and composition.

The action mechanisms for various inhibitory peptides appears to differ depending on the region of the protein used to design the peptide (Koehler et al., 2013), for example, peptides analogous to the hinge region of the fusion protein of dengue virus interfered with the binding to the target cell by prematurely triggering a rearrangement of the viral glycoproteins (Costin et al., 2010), while peptides homologous to the hydrophobic stem region interfered with fusion of the viral and cellular membranes (Schmidt et al., 2010).

To gain some insight into the mechanism by which peptide is able to inhibit virus entry mediated by vp28, interaction of VP28 and the peptide was analyzed by docking and molecular modeling techniques.

### Table 1

| Strains | S. aureus | E. coli | P. vulgaris | S. baltica | V. diabolicus | S. cerevisiae |
|---------|-----------|---------|------------|------------|--------------|--------------|
| DMSO    | –         | –       | –          | –          | –            | –            |
| Peptide solution | + | ++ | + | ++ | + | ++ |

*++ indicated no inhibition zone formation; ‘+’ indicated the inhibition zone was translucent; ‘++’ indicated the inhibition zone was transparent.*
Docking results showed that the peptide could bind with the core β-barrel of VP28 which protruded outside the envelope, and might interact with the host receptor or other envelop proteins, or form trimers, playing a role in virus entry by triggering membrane fusion between the virus and the host, so this peptide may act by interrupting this process. Antiviral assay results could support this point, only the peptide was used in the window period, it could provide protection; if it was degraded, or the shrimp was already infected, it could do nothing.
The core β-barrel of VP28 was highly hydrophobic in nature due to existence of 25 hydrophobic amino acids, so it was easy to establish hydrophobic or Van der Waals interactions with the hydrophobic peptide. Though the predicted N-terminal transmembrane region of VP28 anchored on the viral envelope membrane, which was also hydrophobic, the peptide homologous to this region also has the chance to bind with it if there is a conformational change during fusion. A peptide with a positive WWIHS has the potential to interact with lipid bilayers of the membrane (Melnik et al., 2019). It may also inhibit the viral entry in such a way. Identification of its antimicrobial activity may support this point, this peptide possessed an amphiphilic structure, the obvious characteristic of an antimicrobial peptide. Antimicrobial peptides are a group of membrane active peptides that disrupt the membrane integrity either by pore formation or through nonspecific membrane permeabilization (Lee et al., 2016; Avci et al., 2018). Similarly, this peptide functioning as an antimicrobial peptide also may bind to the similar proteins of used bacteria, which could be concluded from the docking results of the peptide and these proteins (Fig. 5).

As promising templates for developing drugs to occupy a middle space between small molecules and antibodies and for targeting ‘un-druggable’ intracellular protein-protein interactions, peptides have many obvious advantages, such as highly selective and efficacious, relatively safe and well tolerated (Diller et al., 2015), but undeniable, stability and high cost are their main weaknesses, which seriously inhibit the obvious characteristic of an antimicrobial peptide. Antimicrobial peptides are a group of membrane active peptides that disrupt the membrane integrity either by pore formation or through nonspecific membrane permeabilization (Lee et al., 2016; Avci et al., 2018). As for how to use the peptide identified in this work to fight WSSV, in the following study we will manage to build a cyano-bacterial expression system to produce the peptide, which can be applied easily by feeding the shrimp with the genetically modified blue alga.

5. Conclusions

The inhibitory activity of the peptide against WSSV showed that this strategy to design specific peptides targeting important viral envelope proteins through bioinformatics tools was feasible, which provides a potential successful alternative for searching effective anti-WSSV compounds or methods.

Declarations of interest

None.

CRediT authorship contribution statement

Yongzhong Lu: Funding acquisition, Project administration, Writing - original draft. Qian Qiu: Methodology, Data curation. Chen Li: Resources. Linyue Cheng: Validation. Jie Liu: Writing - review & editing.

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