MALAYSIAN JOURNAL OF BIOCHEMISTRY & MOLECULAR BIOLOGY

The Official Publication of The Malaysian Society For Biochemistry & Molecular Biology (MSBMB)
http://mjbmb.org

MULTI-TARGET ANTI-SARS-COV-2 PEPTIDES FROM MEALWORM PROTEINS: AN *IN SILICO* STUDY

Joe-Hui Ong[a], Chieh-Er Liang[a], Wey-Lim Wong[b,c], Fai-Chu Wong[a,c], and Tsun-Thai Chai[a,c,*]

[a]*Department of Chemical Science, Faculty of Science, Universiti Tunku Abdul Rahman, 31900 Kampar, Perak, Malaysia*
[b]*Department of Biological Science, Faculty of Science, Universiti Tunku Abdul Rahman, 31900 Kampar, Perak, Malaysia*
[c]*Center for Agriculture and Food Research, Universiti Tunku Abdul Rahman, 31900 Kampar, Perak, Malaysia*

*Corresponding Author: chaitt@utar.edu.my

History
Received: 11th January 2021
Accepted: 1st April 2021

Keywords:

COVID-19; mealworm; molecular docking; peptide; SARS-CoV-2

Abstract

Peptides are increasingly regarded as promising antagonists to combat Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2). In a recent computational study, we uncovered that mealworm proteins, following *in silico* gastrointestinal digestion, could be a promising source of peptides that potentially block the entry of SARS-CoV-2 into the host cells. In this study, we furthered our investigation to search for mealworm peptides that potentially target SARS-CoV-2 spike glycoprotein, main protease and papain-like protease. Among the 1588 peptide fragments screened, two peptides PKWF and VHRKCF stood out as putative multi-target peptides based on molecular docking analysis. Using *in silico* tools, we also predicted intermolecular interactions that allow binding of the peptides to the target proteins. Relative importance of the individual residues in the two sequences concerning binding stability to the target proteins was investigated. Physicochemical properties of the peptides were also predicted and discussed in relation to their binding to the targets. Overall, our findings suggest that PKWF and VHRKCF could be potential prophylactic or therapeutic agents against SARS-CoV-2. We hope that our findings could pave the way for and benefit future discovery of multi-target anti-SARS-CoV-2 agents from insect proteins, particularly from mealworms.

INTRODUCTION

The ongoing coronavirus disease 2019 (COVID-19), a pneumonia-like pandemic caused by Severe Acute Respiratory Syndrome Coronavirus type 2 (SARS-CoV-2) has drastically affected human life due to high morbidity and mortality [1]. To initiate viral infection, SARS-CoV-2 enters a host cell by interacting with human angiotensin-converting enzyme 2 (hACE2) receptor, mediated by spike glycoprotein receptor-binding domain (RBD). Leu455, Phe456, Ser459, Gln474, Ala475, Phe486, Phe490, Gln493 and Pro499 are the key RBD residues for the binding of SARS-CoV-2 to hACE2 [2]. It has been empirically proved that mutations of the nine aforementioned residues significantly decreased the binding affinity of SARS-CoV-2 towards hACE2 [2]. Following viral entry, main protease (M^{pro}) and papain-like protease (PL^{pro}) of SARS-CoV-2 hydrolyze the viral polyproteins into functional fragments, which are essential for viral replication and transcription [1]. Proteolysis by M^{pro} is mediated by its catalytic-dyad residues His41 and Cys145 [3], whereas that by PL^{pro} is mediated by catalytic-triad residues Cys111, His272 and Asp286 [4]. With the aforementioned, blocking of spike glycoprotein RBD binding to hACE2 and suppression of proteolysis by M^{pro} and PL^{pro} could be the efficacious strategies to suppress SARS-CoV-2 infection, and thus curb the COVID-19 pandemic.

To date, in the search of therapeutics and vaccines for COVID-19 treatment, peptide or peptide-based compounds, namely SBP1, N3 and VIR251, have been experimentally demonstrated as antagonists against SARS-CoV-2 spike glycoprotein RBD, M^{pro} and PL^{pro}, respectively [5-7]. Our

recent computational investigation suggests that following *in silico* gastrointestinal digestion, peptides that potentially block entry of SARS-CoV-2 into the host cells could be released from mealworm proteins [8]. However, whether mealworm-derived peptides could potentially inhibit other SARS-CoV-2 targets, such as M^{pro} and PL^{pro} is unknown. In this study, we investigated multi-target peptides released by *in silico* papain and subtilisin hydrolysis of mealworm proteins, which could serve as antagonists of SARS-CoV-2 spike glycoprotein, M^{pro} and PL^{pro}. Papain and subtilisin were chosen for *in silico* hydrolysis in light of their ability to release bioactive peptides from mealworm proteins [9, 10]. Meanwhile, multi-target therapeutics are preferable as prevention and/or treatment for diseases associated with high-mutation-rate RNA viruses like SARS-CoV-2 [11].

MATERIALS AND METHODS

In silico Hydrolysis of Mealworm Proteins

The sequences of five muscle proteins (actin-like [UniProt ID: S5M0Y7], alpha-actinin-4 [UniProt ID: E0VM19], calponin [UniProt ID: D2A180], tropomyosin 2 [UniProt ID: V5JDH8] and troponin T [UniProt ID: D3TS62]) as well as three non-muscle proteins (12-kDa hemolymph protein B [UniProt ID: Q7YWD7], 13-kDa hemolymph protein A [UniProt ID: Q7YWD2] and 28-kDa desiccation stress protein [UniProt ID: Q27013]) of *Tenebrio molitor* L. (Tenebrionidae) (mealworm) [12] were retrieved from the UniProt database (http://www.uniprot.org) [13]. All the eight proteins were *in silico* hydrolyzed by papain (EC 3.4.22.2) and subtilisin (EC 3.4.21.62) independently on the BIOPEP-UWM bioactive peptides database, using the 'Enzyme(s) action' tool [14].

Molecular Docking Analyses

Mealworm peptides and selected reference peptides were docked against three targets: SARS-CoV-2 spike glycoprotein RBD, M^{pro} and PL^{pro}. The crystal structures of SARS-CoV-2 spike glycoprotein RBD complexed with hACE2 (PDB ID: 6LZG) [15], M^{pro} complexed with N3 (PDB ID: 6LU7) [5] and PL^{pro} complexed with VIR251 (PDB ID: 6WX4) [6] were downloaded from Protein Data Bank (http://www.rcsb.org/pdb) [16, 17]. The bound substrate or ligands (hACE2, N3 and VIR251) were separated from viral proteins (spike glycoprotein RBD, M^{pro} and PL^{pro}) using BIOVIA Discovery Studio Visualizer (BIOVIA, Dassault Systèmes, BIOVIA Discovery Studio Visualizer, Version 20.1.0.192, San Diego: Dassault Systèmes, 2020). Proteins and ligands were prepared for molecular docking as previously described [8]. SBP1 is a natural 23-mer peptide derived from hACE2; its binding affinity towards spike glycoprotein RBD of SARS-CoV-2 was experimentally demonstrated to be comparable with that of full-length hACE2 [7]. For the preparation of SBP1, the region between Ile21 and Ser43 of hACE2 was extracted while the rest of the residues were removed.

The prepared proteins RBD, M^{pro} and PL^{pro} were uploaded as receptor inputs on HPEPDOCK server (http://huanglab.phys.hust.edu.cn/hpepdock) [18], whereas prepared ligands SBP1, N3 and VIR251 were uploaded as binding site references, respectively. Mealworm peptides released by *in silico* hydrolysis and selected reference peptides (Table 1), where crystal structures are not available, were entered in FASTA format as peptide inputs. Reference peptides taken in this study (Table 1) are all previously reported as SARS-CoV-2 inhibitory peptides. The docking scores of peptides computed by HPEPDOCK were recorded. The HPEPDOCK docking score is used as relative ranking among the binding models. Peptides with higher (more negative) docking scores ranked better [18]. A strong correlation between bioactivity of peptides and HPEPDOCK docking scores was reported [19]. Three dimensional (3D) diagrams of viral protein models docked against peptides were visualized by using BIOVIA Discovery Studio Visualizer. Two dimensional (2D) diagrams of docked models with higher (more negative) docking scores relative to SBP1, N3 and/or VIR251 were produced using LigPlot+ v.2.2. [20, 21].

Toxicity, Allergenicity, Bioactive and Antiviral Predictions as well as Physicochemical Properties of Peptides

Toxicity of peptides was predicted by using ToxinPred (http://crdd.osdd.net/raghava/toxinpred). Toxicity prediction method was based on support vector machine (SVM) and the default SVM threshold of 0.0 was chosen [28]. Peptides with SVM score < 0.0 are predicted as non-toxin. Allergenicity prediction was done by using AllerTOP v.2.0 (http://www.ddg-pharmfac.net/AllerTOP) [29]. Potential of peptides being bioactive was computed by using PeptideRanker (http://distilldeep.ucd.ie/PeptideRanker) at a threshold of 0.5. Peptides predicted to have PeptideRanker score over 0.5 threshold is considered as bioactive [30]. Probability (between 0 and 1) of being antiviral was computed by using Meta-iAVP (http://codes.bio/meta-iavp) at the threshold of 0.5 where the peptides predicted with score > 0.5 labelled as antiviral [31]. Physicochemical properties of peptides were predicted by using Peptides Package in R (https://rdrr.io/snippets) [32].

Table 1: Previously reported potential SARS-CoV-2 inhibitory peptides that were used as reference peptides in this study.

Viral proteins	Reference peptides	Nature	Remarks	Source of crystal structure	References
M^{pro}	ALPMHIR	Peptide	Derived from goat milk beta-lactoglobulin	-	[22]
	EEAGGATAAQIEM	Peptide	Derived from tuna skeletal myosin	-	[23]
	IPAVFK	Peptide	Derived from goat milk beta-lactoglobulin	-	[22]
	LPIY	Peptide	Derived from *Mizugopecten yessoensis* myosin	-	[24]
	N3[a]	Peptide-based	Computer-aided drug design	PDB ID: 6LU7	[5]
	p28 (LSTAADMQGVVTDGMASGLDKDYLKPDD)	Peptide	Derived from bacteria azurin	PBD ID: 1E5Z	[25]
	QRPR	Peptide	Derived from *Mizugopecten yessoensis* myosin	-	[24]
PL^{pro}	p18 (LSTAADMQGVVTDGMASG)	Peptide	Derived from bacteria azurin	PBD ID: 1E5Z	[25]
	p28 (LSTAADMQGVVTDGMASGLDKDYLKPDD)	Peptide	Derived from bacteria azurin	PBD ID: 1E5Z	[25]
	VIR250[a]	Peptide-based	Computer-aided drug design	PBD ID: 6WUU	[6]
	VIR251[a]	Peptide-based	Computer-aided drug design	PBD ID: 6WX4	[6]
Spike protein RBD	ALPMHIR	Peptide	Derived from goat milk beta-lactoglobulin	-	[22]
	FLDKFNHEAEDLFYQSSL	Peptide	Derived from hACE2	-	[26]
	IPAVFK	Peptide	Derived from goat milk beta-lactoglobulin	-	[22]
	PISCR	Peptide	Derived from wheat ribulose bisphosphate carboxylase small chain	-	[27]
	PQQQF	Peptide	Derived from barley D hordein	-	[27]
	SBP1 (IEEQAKTFLDKFNHEAEDLFYQS)	Peptide	Derived from hACE2	PDB ID: 6LZG	[7]
	VPW	Peptide	Derived from mealworm alpha-actinin-4	-	[8]
	VQVVN	Peptide	Derived from oat 11S globulin	-	[27]

[a]N3: N-[(5-methylisoxazol-3-yl)carbonyl]alanyl-L-valyl-N~1~-((1R,2Z)-4-(benzyloxy)-4-oxo-1-{[(3R)-2-oxopyrrolidin-3-yl]methyl}but-2-enyl)-L-leucinamide; VIR250: Ac-Abu(Bth)-Dap-Gly-Gly-VME; VIR251: Ac-hTyr-Dap-Gly-Gly-VME

Computational Alanine Scanning

Computational alanine scanning experiment was performed using BUDE Alanine Scan (https://balas.app) [33, 34]. The protein-peptide docked models were uploaded to the server to identify peptide residues that are important for binding with viral proteins. The change in overall binding energy (ΔΔG) resultant from alanine substitution of each residue was recorded. The more positive the ΔΔG value predicted for a residue is, the greater its contribution to stably binding to a viral target [34].

RESULTS AND DISCUSSION

In silico hydrolysis of the eight mealworm proteins by papain and subtilisin yielded 922 and 666 sequence fragments, respectively. The same peptide sequences can be released from the same protein or different proteins. Thus we manually checked the combined pool of 1588 peptide fragments for identical sequences. The exclusion of redundant sequences resulted in a dataset of 850 peptide fragments with unique sequences, ranging from 2 to 41 residues in length. Docking scores of the 850 peptides spread over the range from -33.821 to -246.196, -37.284 to -209.211, and -38.925 to -230.131 corresponding to the analysis on M^{pro}, PL^{pro} and spike glycoprotein RBD, respectively. Out of the 850, 12 have higher (more negative) docking scores relative to N3 and/or VIR251. Out of the 12, 5 are predicted to bind to at least one key binding residue on spike glycoprotein RBD, at least one catalytic-dyad residue of M^{pro} and at least one catalytic-triad residue of PL^{pro} (Table 2). We observed the same with other studies [8, 35, 36], where the interactions between peptides and viral proteins were mostly governed by hydrogen bonds and hydrophobic interactions. The two aforementioned interactions are said to be critical in promoting the binding of the peptide to proteins. Taken together, the five peptides listed in Table 2 were predicted as potential trifunctional antagonists against SARS-CoV-2 spike glycoprotein, M^{pro} and PL^{pro}.

Table 2: Docking scores computed for five potential trifunctional mealworm peptides and their number of interactions with the target proteins.

Proteases	Peptides	Docking scores[a]			Number of key binding/ catalytic residues the peptide interacting with[b]		
		M^{pro}	PL^{pro}	RBD	M^{pro}	PL^{pro}	RBD
Subtilisin	VHRKCF	-199.681	**-209.211**	-164.178	2	2	4
	CQRKTAPY	**-246.196**	-179.322	-162.228	2	2	1
Papain	YVSSYYHT	**-223.011**	**-188.337**	-208.241	2	1	4
	PKWF	-204.309	**-196.988**	-169.865	2	2	2
	AEYCIKR	**-226.324**	-149.529	-165.118	2	1	3

[a]The docking scores in **bold** are higher (more negative) than those predicted for N3 (-215.634) and VIR251 (-186.078), the inhibitors complexed to the M^{pro} and PL^{pro} crystal structures, respectively.

[b]Nine key binding residues critical for binding between spike glycoprotein RBD and hACE2 are Leu455, Phe456, Ser459, Gln474, Ala475, Phe486, Phe490, Gln493 and Pro499. The catalytic dyad in the active site of M^{pro} consists of His41 and Cys145. The catalytic triad in the active site of PL^{pro} consists of Cys111, His272 and Asp286.

Comparison of docking scores of the five mealworm peptides with reference peptides on analysis of M^{pro}, PL^{pro} and spike glycoprotein RBD are shown in Figure 1. Corresponding to the analysis on M^{pro} and PL^{pro}, the docking scores of CQRKTAPY and VHRKCF were 14-53% and 12-43% higher than that of all the reference peptides, respectively (Figures 1A, 1B). Analysis on spike glycoprotein RBD revealed that the docking score of YVSSYYHT was 28-87% higher than that of reference peptides ALPMHIR, IPAVFK, PQQQF, PISCR, VPW and VQVVN (Figure 1C). By contrast, none of the mealworm peptides exceeded the two hACE2-derived reference peptides, SBP1 (-232.680) and FLDKFNHEAEDLFYQSSL (-219.478) in terms of docking scores (Figure 1C).

At present, none of the 16 reference peptides used in this study were previously reported as trifunctional peptides, which could concurrently antagonize the M^{pro}, PL^{pro} and spike glycoprotein RBD of SARS-CoV-2. Hence, for a comparison between the five potential trifunctional mealworm peptides and potential trifunctional reference peptides, we decided to perform molecular docking analysis on all 16 reference peptides against the three viral proteins associated with the viral infection. Our analysis found that six reference peptides, namely FLDKFNHEAEDLFYQSSL, PQQQF, PISCR, VIR251, EEAGGATAAQIEM, and VIR250 are potentially trifunctional. The six peptides were predicted to bind to as least one key binding/catalytic residues of spike protein RBD, M^{pro} and PL^{pro} (data not shown). p28 and VPW are single-functional peptide, predicted to bind to only RBD and M^{pro}, respectively. Meanwhile, the other eight reference peptides (SBP1, IPAVFK, p18, LPIY, VQVVN, N3, QRPR, and ALPMHIR) are bifunctional, predicted to bind to only both M^{pro} and RBD. In this study, when docked against M^{pro}, the docking score of mealworm peptide

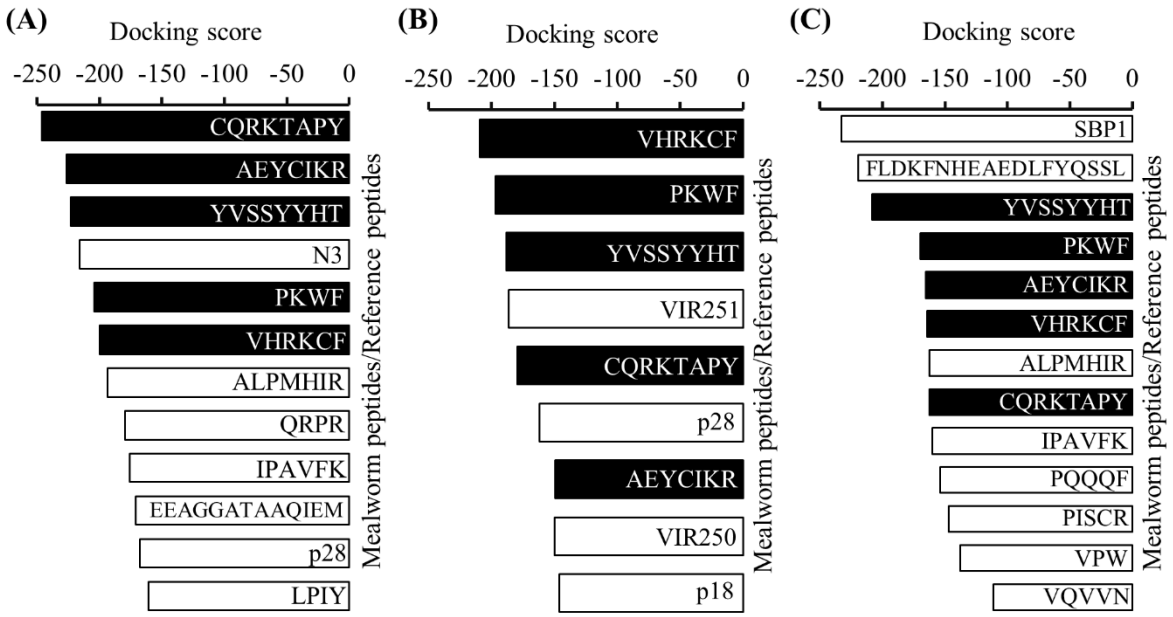

Figure 1: Docking scores of mealworm peptides and reference peptides based on analysis on (**A**) M^{pro}, (**B**) PL^{pro} and (**C**) spike glycoprotein RBD. Black bars represent mealworm peptides, whereas white bars represent reference peptides. Sequences of the peptides are presented inside the bars.

CQRKTAPY is 30-126% higher than those of the six potentially trifunctional reference peptides (PQQQF, FLDKFNHEAEDLFYQSSL, EEAGGATAAQIEM, PISCR, VIR250, and VIR251). Similarly, when docked against PL^{pro}, the docking score of mealworm peptide VHRKCF is 12-58% higher than those of the six trifunctional reference peptides (VIR251, FLDKFNHEAEDLFYQSSL, PQQQF, PISCR, VIR250, and EEAGGATAAQIEM). Meanwhile, our analysis revealed that although SBP1 has a 12% higher docking score than trifunctional mealworm peptide YVSSYYHT when docked against RBD (Figure 1C); SBP1 is only potentially a bifunctional peptide. Interestingly, we found that mealworm peptide YVSSYYHT resembles reference peptides FLDKFNHEAEDLFYQSSL, with which it differs in docking score by 5%. Moreover, both peptides are trifunctional and were predicted to bind to the same number of key binding/catalytic residues in each of the three targets investigated. Taken together, based on our molecular docking analysis, the five trifunctional mealworm peptides are apparently superior to most of the aforementioned reference peptides reported in the literature in terms of their potential to bind to and thus acting as multifunctional antagonists of SARS-CoV-2 targets. Multi-target antagonists are desirable as they are more likely to be effective in tackling the COVID-19 pandemic when compared with single-target antagonists [11].

Among the five mealworm peptides, PKWF and VHRKCF were predicted to be non-toxin and likely non-allergenic with a high probability to be bioactive (0.98 and 0.56) and antiviral (0.96 and 1.00) (Table 3). The mass (576.696 Da and 788.966 Da), net charge (0.976 and 2.185), isoelectric point (9.700 and 9.834) and instability index (7.500 and 8.333) of PKWF and VHRKCF were predicted, respectively. They are basic in nature with basic amino acid composition 25-50%. Such properties

Table 3: Predicted toxicity, allergenicity, probability to be bioactive and antiviral of five potential trifunctional mealworm peptides.

Peptides	Toxicity [SVM score]	Allergenicity	PeptideRanker score	Antiviral prediction[a]
PKWF	Non-toxin [-0.76]	Probable non-allergen	0.9751	AVP [0.962]
VHRKCF	Non-toxin [-0.62]	Probable non-allergen	0.5557	AVP [1.000]
AEYCIKR	Non-toxin [-0.05]	Probable allergen	0.4606	Non-AVP [0.430]
CQRKTAPY	Non-toxin [-0.67]	Probable non-allergen	0.2416	Non-AVP [0.000]
YVSSYYHT	Non-toxin [-0.67]	Probable non-allergen	0.2074	Non-AVP [0.000]

[a]Values in brackets are probability to be AVP. AVP: antiviral peptide

could facilitate their suppression on virus-host endosomal acidification that are essential for releasing viral RNA into host cells [37]. As a positively charged species, they could exhibit higher electrostatic affinities toward negatively-charged viral surface and cause viral envelope disruption [37]. They are considered stable as revealed by their instability index lower than 40 [32]. With the aforementioned, PKWF and VHRKCF could suppress SARS-CoV-2 infection through different route of actions without eliciting toxicity and allergenicity. Their potential as SARS-CoV-2 inhibitory peptides through hampering endosomal acidification and viral envelope disruption remains to be ascertained. Besides, with the extremely high probability predicted to be bioactive and antiviral, whether they could also exhibit other health-promoting effects or broad-spectrum antiviral activities, deserve to be discovered.

Computational alanine scanning on M^{pro} analysis revealed that all the residues in PKWF increased ΔΔG following alanine substitution (Table 4). It indicates that if alanine is substituted for any residues, the compatibility of PKWF with M^{pro} will be diminished. The aforementioned could be attributed to the loss of direct M^{pro}-PKWF binding or change of peptide conformation upon alanine substitution. Remarkably, on the analysis of M^{pro} and spike glycoprotein RBD, alanine substitution of C-terminal Phe residue in both PKWF and VHRKCF drastically increased ΔΔG, making the protein-peptide structure less stable. A similar observation was reported by [26], where the binding of the peptide to spike glycoprotein RBD becomes less compatible upon alanine substitution of N-terminal Phe residue in peptide FLDKFNHEAEDLFYQSSL. It would thus suggest that the terminal Phe is a stabilizing residue for binding of peptides to M^{pro} and spike glycoprotein RBD. Our LigPlot+ analysis also revealed that Phe of PKWF could form hydrogen bonds with key catalytic residue Cys145 of M^{pro} (Figure 2A) and key binding residue Gln493 on spike glycoprotein RBD (Figure 2E). Besides, His of VHRKCF could also be an important residue for binding peptide to PL^{pro}, as it increased ΔΔG by 8.7914 kJ/mol after alanine substitution. It was also predicted to form a hydrogen bond with key catalytic residue Cys111 of PL^{pro} (Figure 2D).

In contrast, substituting Cys of VHRKCF by alanine decreased or had no effect for ΔΔG based on analysis of all the three target proteins. Cys seems to be less important for interacting with viral proteins, although it could form an external bond and hydrogen bond with His41 and Cys145 of M^{pro}, respectively (Figure 2B). Our finding suggests that if alanine is substituted for Cys, VHRKCF will exhibit stronger binding affinity towards the three viral proteins. However, since Cys could interact with catalytic-dyad residues of M^{pro}, whether it is possible to substitute Cys with alanine and still maintain the binding of VHRKCF to these residues, remains to be determined.

Table 4: Computational alanine scanning of residues in two promising trifunctional mealworm peptides.

Peptides	Residues	ΔΔG (kJ/mol)		
		M^{pro}	**PL^{pro}**	**Spike protein RBD**
PKWF	Pro	2.3981	-0.3104	2.0004
	Lys	1.2333	2.4101	2.3527
	Trp	6.6196	7.8602	-9.5418
	Phe	10.0598	3.2054	7.9935
VHRKCF	Val	5.6526	3.1373	0.9486
	His	1.5846	8.7914	1.4374
	Arg	9.5701	17.1142	3.1868
	Lys	9.6920	3.1485	2.9085
	Cys	-4.8950	-0.2348	0
	Phe	10.4596	-1.8939	16.9808

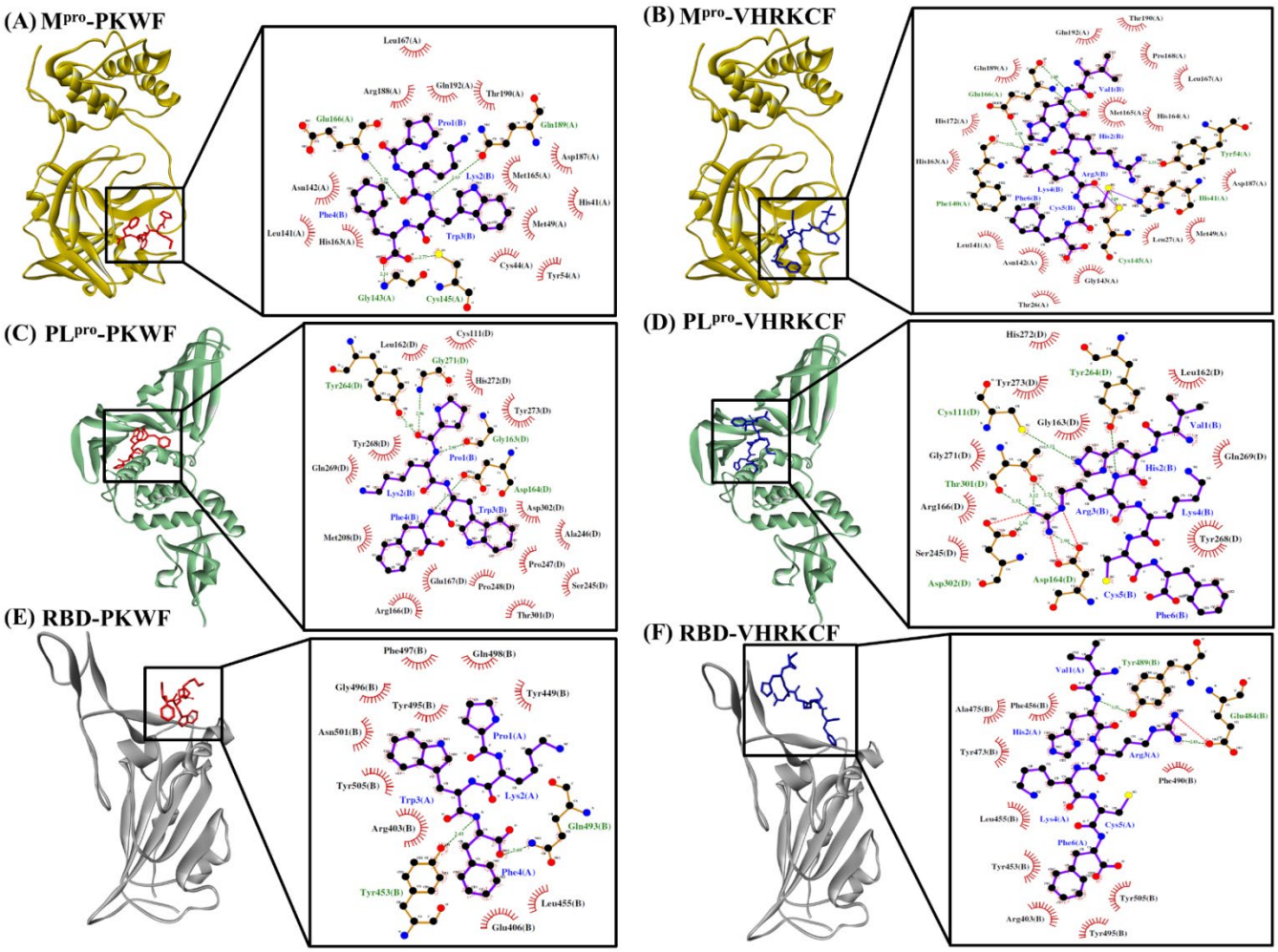

Figure 2: The docked models of PKWF against (**A**) M^{pro}, (**C**) PL^{pro} and (**E**) spike glycoprotein RBD, as well as those of VHRKCF against (**B**) M^{pro}, (**D**) PL^{pro} and (**F**) spike glycoprotein RBD are presented in 3D (left) and 2D (right) diagrams. In the 3D diagram, PKWF and VHRKCF structures are displayed in red and blue, respectively. In the 2D diagram, bonds of proteins are in orange, whereas that of peptides are in purple. Hydrophobic interactions, external bonds, hydrogen bonds and salt bridges are represented in red spoked arcs, purple lines, green and red dashed lines, respectively. The projected view displays the binding interface region between the proteins and peptides.

Overall, two mealworm protein-derived peptides PKWF and VHRKCF could serve as putative multi-target prophylactic or therapeutic agents against SARS-CoV-2. We believed that our findings could benefit future research and development of multi-target anti-SARS-CoV-2 peptides against M^{pro}, PL^{pro} and spike glycoprotein RBD. Future *in vitro* and *in vivo* validations of the potency of the two peptides are warranted.

ACKNOWLEDGMENT

We thank Jia-Ying Lim for her assistance in data collection.

CONFLICT OF INTEREST

The authors declare no financial or commercial conflict of interest.

REFERENCES

1. Haque, S.K.M., Ashwaq, O., Sarief, A. and John, A.K.A. (2020) A comprehensive review about SARS-CoV-2. Future Virol. **15**(9), 625-648.

2. Yi, C., Sun, X., Ye, J., Ding, L., Liu, M., Yang, Z., Lu, X., Zhang, Y., Ma, L., Gu, W., Qu, A., Xu, J., Shi, Z., Ling, Z. and Sun, B. (2020) Key residues of the receptor binding motif in the spike protein of SARS-CoV-2 that interact with ACE2 and neutralizing antibodies. Cell Mol. Immunol. **17**, 621-630.

3. Ramos-Guzmán, C.A., Ruiz-Pernía, J.J. and Tuñón, I. (2020) Unraveling the SARS-CoV-2 main protease mechanism using multiscale methods. ACS Catal. **10**(21), 12544-12554.

4. Wlodawer, A., Dauter, Z., Shabalin, I.G., Gilski, M., Brzezinski, D., Kowiel, M., Minor, W., Rupp, B. and Jaskolski, M. (2020) Ligand-centered assessment of SARS-CoV-2 drug target models in the Protein Data Bank. FEBS J. **287**(17), 3703-3718.

5. Jin, Z., Du, X., Xu, Y., Deng, Y., Liu, M., Zhao, Y., Zhang, B., Li, X., Zhang, L., Peng, C., Duan, Y., Yu, J., Wang, L., Yang, K., Liu, F., Jiang, R., Yang, X., You, T., Liu, X., Yang, X., Bai, F., Liu, H., Liu, X., Guddat, L.W., Xu, W., Xiao, G., Qin, C., Shi, Z., Jiang, H., Rao, Z. and Yang, H. (2020) Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature **582**, 289-293.

6. Rut, W., Lv, Z., Zmudzinski, M., Patchett, S., Nayak, D., Snipas, S.J., El Oualid, F., Huang, T.T., Bekes, M., Drag, M. and Olsen, S.K. (2020) Activity profiling and crystal structures of inhibitor-bound SARS-CoV-2 papain-like protease: A framework for anti-COVID-19 drug design. Sci. Adv. 6, eabd4596.

7. VanPatten, S., He, M., Altiti, A., F Cheng, K., Ghanem, M.H. and Al-Abed, Y. (2020) Evidence supporting the use of peptides and peptidomimetics as potential SARS-CoV-2 (COVID-19) therapeutics. Future Med. Chem. **12**(18), 1647-1656.

8. Wong, F.-C., Ong, J.-H. and Chai, T.-T. (2020) Identification of putative cell-entry-inhibitory peptides against SARS-CoV-2 from edible insects: An *in silico* study. eFood **1**(5), 357-368.

9. Dai, C., Ma, H. and Luo, L. (2013) Angiotensin I-converting enzyme (ACE) inhibitory peptide derived from *Tenebrio molitor* (L.) larva protein hydrolysate. Eur. Food Res. Technol. **236**, 681-689.

10. Sun, A.-d., Cui, N., Zhu, Y., Zhu, N., Li, F.-m. and Sun, J.-q. (2019) Preparation of ACE inhibitory pepides from *Tenebrio molitor* and antibacterial activity. SDRP J. Food Sci. Technol. **4**(3), 670-679.

11. Pachetti, M., Marini, B., Benedetti, F., Giudici, F., Mauro, E., Storici, P., Masciovecchio, C., Angeletti, S., Ciccozzi, M., Gallo, R.C., Zella, D. and Ippodrino, R. (2020) Emerging SARS-CoV-2 mutation hot spots include a novel RNA-dependent-RNA polemerase variant. J. Transl. Med. **18**, 179.

12. Yi, L., Van Bookel, M.A.J.S., Boekel, S. and Lakemond, C.M.M. (2016) Protein identification and in vitro in digestion of fractions from *Tenebrio molitor*. Eur. Food Res. Technol. **242**, 1285-1297.

13. The UniProt Consortium. (2019) UniProt: a worldwide hub of protein knowledge. Nucleic Acids Res. **47**(D1), D506-D515.

14. Minkiewicz, P., Iwaniak, A. and Darewicz, M. (2019) BIOPEP-UWM database of bioactive peptides: Current opportunities. Int. J. Mol. Sci. **20**(23), 5978.

15. Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., Lu, G., Qiao, C., Hu, Y., Yuen, K.-Y., Wang, Q., Zhou, H., Yan, J. and Qi, J. (2020). Structural and functional basis of SARS-CoV-2 entry by using human ACE2. Cell **181**(4), 894-904.

16. Berman, H.M., Westbrook, J., Feng, Z., Gilliland, G., Bhat, T.N., Weissig, H., Shindyalov, I.N. and Bourne, P. E. (2000). The Protein Data Bank. Nucleic Acids Res. **28**, 235-242.

17. Burley, S.K., Berman, H.M., Bhikadiya, C., Bi, C., Chen, L., Di Costanzo, L., Christie, C., Dalenberg, K., Duarte, J.M., Dutta, S., Feng, Z., Ghosh, S., Goodsell, D.S., Green, R.K., Guranović, V., Guzenko, D., Hudson, B.P., Kalro, T., Liang, Y., Lowe, R., Namkoong, H., Peisach, E., Periskova, I., Prlić, A., Randle, C., Rose, A., Rose, P., Sala, R., Sekharan, M., Shao, C., Tan, L., Tao, Y.-P., Valasatava, Y., Voigt, M., Westbrook, J., Woo, J., Yang, H., Young, J., Zhuravleva, M. and Zardecki, C. (2018) RCSB Protein Data Bank: biological macromolecular structures enabling research and education in fundamental biology, biomedicine, biotechnology and energy. Nucleic Acids Res. **47**(D1), D464-D474.

18. Zhou, P., Jin, B., Li, H. and Huang, S.Y. (2018) HPEPDOCK: a web server for blind peptide-protein docking based on a hierarchical algorithm. Nucleic Acids Res. **46**(W1), W443-W450.

19. Yang, X., Wang, K., Liu, Q. and Zhang, X. (2020) Discovery of monoamine oxidase A inhibitory peptides from hairtail (*Trichiurus japonicus*) using *in vitro* simulated gastrointestinal digestion and *in silico* studies. Bioorg. Chem. **101**, 104032.

20. Laskowski, R.A. and Swindells, M.B. (2011) LigPlot+: multiple ligand-protein interaction diagrams for drug discovery. J. Chem. Inf. Model. **51**(10), 2778-2786.

21. Wallace, A.C., Laskowski, R.A. and Thornton, J.M. (1995) LIGPLOT: a program to generate schematic diagrams of protein-ligand interactions. Protein Eng. Des. Sel. **8**(2), 127-134.

22. Çakır, B., Okuyan, B., Şener, G. and Tunali-Akbay, T. (2021) Investigation of beta-lactoglobulin derived bioactive peptides against SARS-CoV-2 (COVID-19): *In silico* analysis. Eur. J. Pharmacol. **891**(15), 173781.

23. Yu, Z., Kan, R., Ji, H., Wu, S., Zhao, W., Shuian, D., Liu, J. and Li, J. (2021) Identification of tuna protein-derived peptides as potent SARS-CoV-2 inhibitors via molecular docking and molecular dynamic simulation. Food Chem. 342, 128366.

24. Bu, Y., He, W., Wang, F., Zhu, W., Li, X., Yi, S., Xu, Y. and Li, J. (2020) Screening research of anti-SARS-CoV-2 peptides from *Mizuhopecten yessoensis* based on molecular docking. J. Food Sci. Technol. (China) **38**(4), 54-62.

25. Sasidharan, S., Selvaraj, C., Singh, S.K., Dubey, V.K., Kumar, S., Fialho, A.M. and Saudagar, P. (2020) Bacterial protein azurin and derived peptides as potential anti-SARS-CoV-2 agents: insights from molecular docking and molecular dynamics simulation. J. Biomol. Struct. Dyn. https://doi.org/10.1080/07391102.2020.1787864

26. Baig, M.S., Alagumuthu, M., Rajpoot, S. and Saqib, U. (2020) Identification of a potential peptide inhibitor of SARS-CoV-2 targeting its entry into the host cells. Drugs R D. **20**(3), 161-169.

27. Luo, Z., Su, K. and Zhang, X. (2020) Potential of plant proteins digested *in silico* by gastrointestinal enzymes as nutritional supplement for COVID-19 patients. Plant Foods Hum. Nutr. **75**, 583-591.

28. Gupta, S., Kapoor, P., Chaudhary, K., Gautam, A., Kumar, R., Open Source Drug Discovery, C. and Raghava, G.P.S. (2013) *In silico* approach for predicting toxicity of peptides and proteins. PLOS ONE **8**(9), e73957.

29. Dimitrov, I., Bangov, I., Flower, D.R. and Doytchinova, I. (2014) AllerTOP v.2--a server for *in silico* prediction of allergens. J. Mol. Model. **20**(6), 2278.

30. Mooney, C., Haslam, N.J., Pollastri, G. and Shields, D.C. (2012) Towards the improved discovery and design of functional peptides: common features of diverse classes permit generalized prediction of bioactivity. PLOS ONE **7**(10), e45012.

31. Schaduangrat, N., Nantasenamat, C., Prachayasittikul, V. and Shoombuatong, W. (2019) Meta-iAVP: A sequence-based meta-predictor for improving the prediction of antiviral peptides using effective feature representation. Int. J.Mol. Sci. **20**(22), 5743.

32. Osorio, D., Rondón-Villarreal, P. and Torres, R. (2015) Peptides: A package for data mining of antimicrobial peptides. R J. **7**(1), 4-14.

33. Ibarra, A.A., Bartlett, G.J., Hegedüs, Z., Dutt, S., Hobor, F., Horner, K.A., Hetherington, K., Spence, K., Nelson, A., Edwards, T.A., Woolfson, D.N., Sessions, R.B. and Wilson, A.J. (2019) Predicting and experimentally validating hot-spot residues at protein–protein interfaces. ACS Chem. Biol. **14**(10), 2252-2263.

34. Wood, C.W., Ibarra, A.A., Bartlett, G.J., Wilson, A.J., Woolfson, D.N. and Sessions, R.B. (2020) BAlaS: fast, interactive and accessible computational alanine-scanning using BudeAlaScan. Bioinformatics **36**(9), 2917-2919.

35. Chowdhury, S.M., Talukder, S.A., Khan, A.M., Afrin, N., Ali, M.A., Islam, R., Parves, R., Al Mamum, A., Sufian, M.A., Hossain, M.N., Hossain, M.A. and Halim, M.A. (2020) Antiviral peptides as promising therapeutics against SARS-CoV-2. J. Phys. Chem. B. **124**(44), 9785-9792.

36. Wong, F.-C., Ong, J.-H. and Chai, T.-T. (2021) SARS-CoV-2 spike protein-, main protease- and papain-like-protease-targeting peptides from seed proteins following gastrointestinal digestion: An *in silico* study. Phytomedicine Plus **1**(1), 100016.

37. Mahendran, A.S.K., Lim, Y.S., Fang, C.-M., Loh, H.-S. and Le, C.F. (2020) The potential of antiviral peptides as COVID-19 therapeutics. Front. Pharmacol. **11**, 575444.