Theoretical studies of plant-based peptides targeting human angiotensin converting enzyme-related carboxypeptidase

Babatunde Joseph Oso[1], Ige Francis Olaoye[2] and Adepeju Aberuagba[3]

[1]Department of Biochemistry, McPherson University, Seriki Sotayo, Ogun State, Nigeria
[2]School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool
[3]Department of Biochemistry, University of Ilorin, Ilorin, Nigeria
Correspondence: Ige Francis Olaoye, F.I.Olaoye@ljmu.ac.uk; https://orcid.org/0000-0003-0531-169
Babatunde Joseph Oso, https://orcid.org/0000-0001-8667-7108
Adepeju Aberuagba, https://orcid.org/0000-0003-1055-1407

ABSTRACT

Background

The vulnerability of the lungs, intestine, heart and kidney to SARS-CoV-2 invasion is dependent on the high expression of angiotensin converting enzyme-related carboxypeptidase (ACE2) on the outer surface of the cells in these organs. This clear mode of interaction between SARS-CoV-2 spike proteins and ACE2 emphasizes the importance of ACE2 receptors in the spread of coronaviruses. This study investigated the binding potentials of some selected plant-based peptides (circulin A, kalata B1, Varv peptide E, palicourein, Vhl-1, griffithsin, cycloviolacin VY1) to ACE2 as a predictive approach in preventing SARS-CoV-2 invasion.

Methods

The peptides were retrieved from the antimicrobial peptide database and their respective physicochemical properties were predicted using ProtParam Tool. The binding mode and the binding free energies were computed through HawkDock servers while the structural flexibility and stability of the ACE2-peptide complexes were evaluated via the CABS-flex 2.0 server.

Results

It was observed that the binding scores for the peptides towards ACE2 showed good binding affinities with griffithsin having the best binding score through the Hawkdock rank while kalata B1 had the lowest binding score. The Molecular Mechanics/Generalized Born Surface Area analysis showed that the binding free energy ranges -39.99 and -3.96 kcal/mol with Vhl-1 having the highest free energy and palicourein having the least free energy.

Conclusions

The results of the study suggest that the selected plant-based peptides especially kalata B1, vhl-1, and cycloviolacin VY1 could be promising modulators of ACE2 and prevent the binding of the S1 domain of the SARS-CoV-2 S protein and consequent cellular entry of SARS-CoV-2.

Keywords: Peptides, ACE2, Binding affinity, Modulatory potential, SARS-COV.

INTRODUCTION

More than a decade ago, a novel coronavirus that infects humans, bats and certain other mammals, termed Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV), caused an epidemic with ~ 10% case fatality, creating global panic and economic damage (Lu *et al*., 2014). Recently, another strain of the virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), caused an infectious disease (COVID-19) in human which was for the first time detected in Wuhan, China (Zhang *et al*., 2020). Since then, person to person transmission of the

Received on 9/6/2020 and Accepted for Publication on 17/4/2021.

infectious disease has been on the increase, causing a global pandemic with over 3,946,000 confirmed cases and more than 271,600 deaths (ECDC, 2020). Presently, there is no specific therapies available for the treatment of COVID-19. Social distancing, patient isolation and supportive medical care make up the current management for this infectious disease (Zhang *et al*., 2020).

Coronaviruses use spike proteins (type 1 transmembrane glycoproteins) to mediate viral infection; the subunits of the spike proteins are used to achieve viral fusion and entry (Xia *et al*., 2019). These spike proteins are made up of two subunits, S1 and S2, coronaviruses bind to cellular receptor through S1 subunit's receptor binding domain (RBD), this results in conformational changes of the S2 subunit that leads to the insertion of fusion peptide into the host cell membrane (Xia *et al*., 2019). The amino acid sequencing of the original SARS-CoV and SARS-CoV-2 spike proteins showed that they share 76.5% identity, also, computer modeling of these spiked proteins revealed identical 3D RBD structures (Xu *et al*., 2020). Similar to SARS-CoV and other coronaviruses, SARS-CoV-2 is able to use human angiotensin converting enzyme-related carboxypeptidase (hACE2) as a receptor to infect human cells (Wu *et al*., 2012; Xia *et al*., 2020). Reports from analysis showed that glutamine (residue 394) of SARS-CoV-2 RBD is recognized by lysine (residue 31) on hACE2 receptor, further analysis revealed that SARS-CoV-2 spike proteins recognizes hACE2 more efficiently than SARS-CoV, this is suggested as the reason for the increase in person to person transmission (Wan *et al*., 2020; Zhang *et al*., 2020).

ACE2 is a type 1 integral membrane glycoprotein that is expressed and attached to the outer surface of cells in the lungs, heart, kidney and intestine (Yan *et al*., 2020). ACE2 plays important role in maintaining the renin-angiotensin-aldosterone system (RAAS) balance by catalyzing the cleavage of angiotensin II (vasoconstrictor peptide) into angiotensin 1-7 (vasodilator peptides) (Yan *et al*., 2020). Alveolar epithelial type II cells have been reported to have a high expression of ACE2, about 83%, this suggests that these cells are potential reservoirs for viral invasion making the lung the most vulnerable organ target (Zhang *et al*., 2020). Hashimoto *et al*. (2012) also reported high expression of ACE2 receptors on the luminal surface of intestinal epithelial cells, where they function additionally as co-receptors for amino acid resorption from food. Zhang *et al*. (2020) proposed that the intestine might also be a major entry site for SARS-CoV-2 and that eating food from Wuhan market might have initiated the outbreak of COVID-19 in China. Furthermore, the broad tissue distribution of ACE2 in organs explains the multi-organ dysfunction observed in patients with severe forms of COVID-19 (Huang *et al*., 2020). It has also been speculated that the severity of the infectious disease in the elderly, especially those with cardiovascular comorbidities is influenced by the use of RAAS blockers that increase the expression of ACE2 (Zheng *et al*., 2020). This has led to the hypothesis that decreasing the levels of ACE2 in cells might help in fighting the spread of COVID-19, however ACE2 also play major role in protecting the lung from virus induced injury by increasing the production of angiotensin 1-7 (Tikellis and Thomas, 2012). Also, it is worthy of note that the binding of spike proteins to ACE2 (especially in the lungs) results in the reduced expression and enzymatic activity of ACE2, due to enhanced internalization and may contribute to lung damage that is observed in severe cases of COVID-19 (Jia, 2016).

In a quest for an effective treatment for COVID-19, several potential therapeutic approaches have been proposed such as inhibition of heptad repeat 1 (HR1) in the S2 subunit, inhibition of transmembrane protease serine 2 activity, inhibition of viral six-helical bundle (6-HB) formation, blocking ACE2 receptor, delivering excessive soluble form of ACE2 and spike protein-based vaccine (Yu *et al*., 2016; Zhang *et al*., 2020). The mode of interaction between SARS-CoV-2 spike proteins RBD and ACE2 emphasizes the importance of ACE2 receptors in the spread of COVID-19. Several antiviral drugs have been

proposed as repurposing drugs to impair the binding and replication of the virus; however, these agents might not be healthy alternatives as besides being expensive they produce a wide spectrum of adverse effects. Therefore, this study attempts to investigate the binding potentials of selected plant-based peptides to ACE2

Materials and Methods

Ab Initio modelling of the selected peptides

The 3D structures of the selected peptides were obtained from the Protein Data Bank (https://www.rcsb.org/). The 3D structures of cycloviolacin VY1 and varv peptide E were predicted by submitting their respective FASTA amino acid sequence into I-TASSER (https://zhanglab.ccmb.med.umich.edu/I-TASSER/), an automated modelling server (Yang *et al*., 2015; Zhang *et al*., 2017). The best model for each peptide was selected based on the C-scores and was further validated using the Structure Analysis and Verification server SAVES v5.0 (https://servicesn.mbi.ucla.edu/SAVES/) (Pontius *et al*., 1996).

Physicochemical characterization

The physicochemical characterization of the respective **peptides** was predicted using ProtParam Tool on the SIB ExPASy webserver ProtParam tool (https://www.expasy.org/tools/) using mammalian as the defined organism (Artimo *et al*., 2012). The computed parameters include the molecular weight, theoretical pI, estimated half-life, instability index, aliphatic index, and grand average of hydropathicity.

Database screening

The database screening of peptides with anti-viral properties was conducted using APD3 database (http://aps.unmc.edu/AP/main.php) (Wang *et al*., 2016). The selections were based on the reported biological activities a presented in the APD3 database.

Molecular docking

The structure of native human angiotensin converting enzyme-related carboxypeptidase (ACE2) was retrieved from the Protein Data Bank (https://www.rcsb.org/) with the PDB ID: IR42. The structural bioinformatics studies of the selected peptides with the human ACE2 were computed using the HawkDock server (http://cadd.zju.edu.cn/hawkdock/) integrated with the ATTRACT docking algorithm, the HawkRank scoring function and the Molecular Mechanics/Generalized Born Surface Area (MM/GBSA) free energy decomposition analysis (Feng *et al*., 2017; Weng *et al*., 2019) and the interactions were visualized using PyMOL ver. 1.1eval (De Lano Scientific LLC, CA, USA). All the peptides and the protein were converted into Auto Dock Pdbqt format. The binding energy between the peptides and the protein were computed.

Binding Mode of Docked Complexes

Interactions within the docked complexes were investigated through the Protein Interactions Calculator webserver (http://pic.mbu.iisc.ernet.in/) using the docked complexes (Tina *et al*., 2007). The hydrophobic Interactions, disulphide bridges, hydrogen bonds and ionic Interactions were determined.

Molecular Dynamics (MD) Simulations

The molecular dynamics simulations were carried out using the CABS-flex 2.0 server to evaluate the structural flexibility and stability within a nanosecond time scale of the ACE2-peptide complexes (Kuriata *et al*., 2018). The root-mean-square fluctuations and contact maps were obtained.

Results and Discussion

Ab Initio modelling of the selected peptides

The design of new antiviral molecules is a worldwide priority, especially for the current pandemic viral disease known as COVID-19 due to no specific vaccine or

treatment. The present study employed computational study, as a predictive approach in the drug design. The theoretical models employed for the selection of effective receptor-binding ligands for ACE2 resulted in seven short-listed plant-based peptides belonging to different families of plant species. Natural products of plants origin are considered good chemopreventive agents because of their low toxicities and potential efficacies (Crowell, 2005). Moreover, peptides had been identified to show diverse therapeutic potentials such as antiviral, anticancer, antimicrobial, antiparasitic, etc (Mehta *et al*., 2014; Mustafa *et al*., 2019). Peptides have been identified as effective receptor-binding ligand (Roxin and Zheng, 2012). The selected peptides AP3 ID, peptide names, PDB ID, FASTA sequence, length code, and the corresponding natural sources are presented in Table 1.

Table 1: Identifications of the selected compounds

AP3 ID	Name	PDB ID	FASTA sequence	Length	Source
AP00274	Circulin A	1BH4	GIPCGESCVWIPCISAAL GCSCKNKVCYRN	30	*Chassalia parviflora*
AP00729	Kalata B1	1K48	GLPVCGETCVGGTCNTP GCTCSWPVCTRN	29	*Oldenlandia affinis*
AP01030	Varv peptide E	N/A	GLPICGETCVGGTCNTP GCSCSWPVCTRN	31	*Viola arvensis*
AP01034	Palicourein	1R1F	GDPTFCGETCRVIPVCTY SAALGCTCDDRSDGLCK RN	37	*Palicourea condensata*
AP01058	Vhl-1	1ZA8	SISCGESCAMISFCFTEVI GCSCKNKVCYLN	31	*Viola hederaceae*
AP02133	Griffithsin	2GTY	SLTHRKFGGSGGSPFSGL SSIAVRSGSYLDAIIIDGV HHGGSGGNLSPTFTFGS GEYISNMTIRSGDYIDNI SFETNMGRRFGPYGGSG	121	*Griffithsia* sp
AP02571	Cycloviolacin VY1	N/A	GSANTLSNVKVIQINGSA GDYLDSLDIYYEQY CGESCVFIPCITTVLGCS CSIKVCYKNGSIP	31	*Viola yedoensis*

N/A=Not available

Physicochemical characterization

The result of the predicted physicochemical characterization (Table 2) shows that palicourein, circulin A and kalata B1 has higher and better half-life (30 h) than either Vhl-1 and Griffithsin (1.9 h) or cycloviolacin VY1 and Varv peptide E (1.2 h) that may be due to amino acid composition and cyclic property of the peptides (Vlieghe *et al*., 2010) suggesting that palicourein, circulin A and kalata B1 could resist the proteases degradation and express high bioavailability. The result is in agreement with Mathur *et al*. (2018) who reported that susceptible enzymatic degradation of peptides due to short half-life

reduces their bioavailability and blocked their therapeutic development despite their effective potency over small drugs. However, Vhl-1 and cycloviolacin VY1 bioavailability could be improved either by D-amino acid incorporation (Vlieghe *et al*., 2010) or α-aminoxy amino acid incorporation (Chen *et al*., 2011). The instability index of the peptides was evaluated and the result indicates that Varv peptide E, circulin A, cycloviolacin VY1 and griffithsin have a better instability value (<40) while palicourein and Vhl-1 high instability value and kalata B1 could be slightly stable with instability value of 46.59. Also, the predictive assessment of aliphatic index reveal that varv peptide E, circulin A, Vhl-1, cycloviolacin VY1 and griffithsin have high aliphatic index suggesting the presence of high portion of aliphatic residues such as Leu, Ile and Val which could be responsible for the antiviral potential of the peptides as reported by Chang and Yang (2013). Moreover, the aliphatic index correlates with the instability index except Vhl-1 with moderate high instability index.

Furthermore, the negative GRAVY score result of palicourein and griffithsin indicates their hydrophilic potency suggesting high absorption, ease metabolism and less toxicity but their membrane permeability is slightly poor. However, varv peptide E, circulin A, vhl-1 and cycloviolacin VY1 exhibit better lipophilicity potential due to positive GRAVY score suggesting their ease membrane permeation with difficult solubility and metabolism. This implies that varv peptide E, circulin A, vhl-1 and cycloviolacin VY1 are likely to be toxic thus the intake concentration should be reduced. Interestingly the GRAVY score for kalata B1 is close to zero indicating a balance between the hydrophilic and lipophilic potential of the peptide which suggests a better absorption, metabolism, permeability but less toxic.

Table 2: Physicochemical characterization of the selected peptides

Name	Mol wt (Da)	Theoretical pI	Half-life (h)	Instability index	Aliphatic index	*GRAVY
Circulin A	3175.78	8.33	30	26.56	78.00	0.417
Kalata B1	2916.34	5.96	30	46.59	43.45	0.152
Varv peptide E	3225.88	7.77	1.2	17.44	90.97	0.874
Palicourein	3928.43	4.78	30	60.26	52.70	-0.189
Vhl-1	3340.94	5.85	1.9	56.12	72.26	0.690
Griffithsin	12690.85	5.39	1.9	39.86	70.91	-0.240
Cycloviolacin VY1	3225.88	7.77	1.2	17.44	90.97	0.874

*GRAVY= Grand average of hydropathicity

Binding energy

The Hawkdock server generated 10 predictive models for each of the protein-peptide complex with distinct score values. The first model of each interaction was selected for *in silico* characterization being least value which suggests a model with a high confidence was selected as a final model. The binding scores of the selected peptides after docking with ACE2 are listed in Table 3. The result of the comparative analysis reveals that the binding affinity of the peptides decreased in this order: griffithsin>palicourein>cycloviolacin VY1>vhl-1>circulin A>varv peptide E>kalata B1 with respective binding scores -5314.19>-4215.77>-3987.52>-3690.70>-3637.79>-3585.30>-3280.71. However, studies have shown that docking scores are not satisfactorily presenting the accurate protein-ligand binding affinity (Suenaga *et al*., 2012). Further analysis was performed to predict the free binding energies of the protein–peptide docked

complexes through the MM/GBSA analysis. The MM/GBSA predicted free energies reveals that Vhl-1 had the highest binding free energy (-39.99 kcal/mol) followed by Cycloviolacin VY1 (-38.31 kcal/mol) and kalata B1(-37.49 kcal/mol). Palicourein had the lowest bind free energy followed by griffithsin with values of -3.96 and -19.74 kcal/mol, respectively. The scatter plot obtained from the values of binding free energies of the peptides using the MM/GBSA rescoring against the binding affinity derived from the molecular docking indicates significant discrepancy with low correlation (Figure 1). Thus, the binding affinity could not be efficient for ranking the binding energies as indicated by a small correlation coefficient. The illustrations of the interaction of ACE2 with peptides are shown in Figure 2 along with their corresponding interacting residues identified through the Protein Interactions Calculator server. Three noticeable binding sites were identified in the ACE2 structure. Circulin A, kalata B1, varv peptide E, vhl-1, and cycloviolacin VY1 were revealed to occupy the major binding site on ACE2 while palicourein and griffithsin were bound at different pockets.

Table 3: Binding scores and the MM-GBSA free binding energy

Name	HawkRank Scores	MM/GBSA free energy (kcal/mol)
Circulin A	-3637.79	-26.33
Kalata B1	-3280.71	-37.49
Varv peptide E	-3585.30	-31.26
Palicourein	-4215.77	-3.96
Vhl-1	-3690.70	-39.99
Griffithsin	-5314.19	-19.74
Cycloviolacin VY1	-3987.52	-38.31

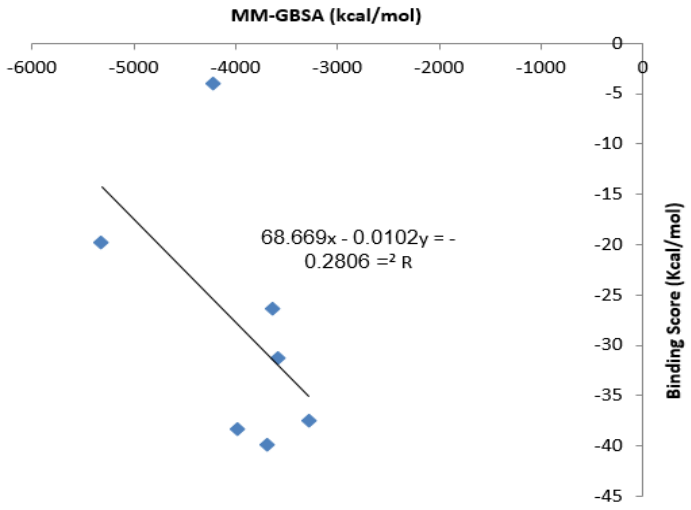

Figure 1: The scatter plot of MM/GBSA binding free energy versus binding affinity.

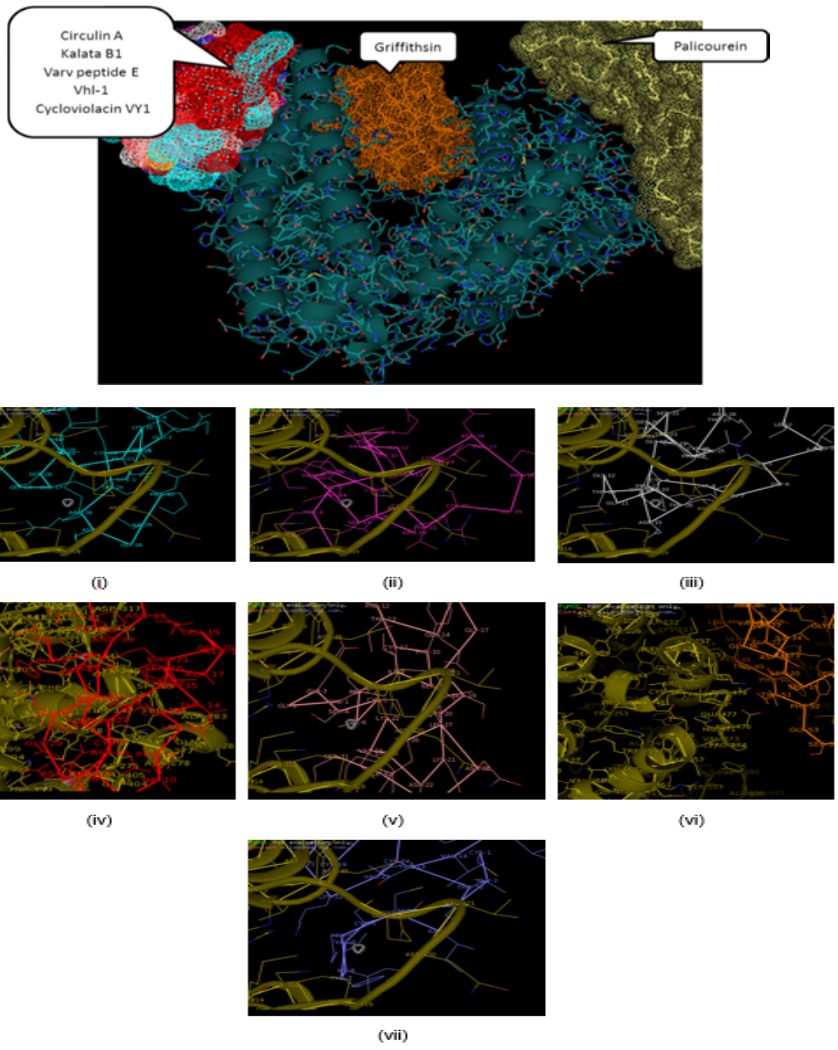

Figure 2: 3D illustrations of (A) the binding positions and (B) interacting residues of complex of ACE2 with (i) Circulin A, (ii) Kalata B1, (iii) Varv peptide E, (iv) Palicourein, (v) Vhl-1, (vi) Griffithsin, (vii) Cycloviolacin VY1

ACE2-peptide interactions

The interactions of the ACE2 with the peptides are presented in Tables 4 and 5. The main hydrophobic contacts within 5 Angstroms were established through ALA-28, MET-314, and PRO-318 in ACE2 and PHE-12, and ILE-30 in Vhl-1. TRP-30, TYR-23, and LEU-27 located in the main binding pocket of ACE2 contributed to the hydrophobic interactions involving PRO-9 and ILE-21 of cycloviolacin VY1 (Table 4). In addition, the hydrophobic interactions were established through PRO-271 and VAL-346 of ACE2 and VAL-9 and TYR-15 in palicourein while they occurred between ACE2 and griffithsin through VAL-154, PRO-117, TRP-145, and PRO-120, and LEU-46, PRO-48, and TYR-85, respectively. The predicted interaction also showed that TYR-23, LEU-27, TRP-30, and MET-314 from the ACE2 could be considered key interactions residues due to their frequent occurrences. There was no protein-protein disulphide bridges found in the interactions of the peptides with ACE2. However, ionic interaction within 6 A˚ were established in palicourein, griffithsin, and cycloviolacin

VY1with ACE2 while aromatic-aromatic interaction within 4.5 and 7 A˚ were found in theACE2 interactions with kalata B1 and vhl-1. Interactions through hydrogen bonding were found to exist between ACE2 and the peptides. These parameters demonstrated the binding strength between ACE2 and the peptides.

Table 4: Possible residues involving in the interactions of ACE2 and the peptides

Peptides	Hydrophobic Interactions*		Ionic Interactions#		Aromatic-Aromatic Interactions§	
	AR	PR	AR	PR	AR	PR
Circulin A	ALA-28	PRO-9				
	ALA-47	PRO-9				
	TYR-23	ALA-14				
	LEU-27	ALA-14				
	TYR-23	LEU-15				
Kalata B1	MET-314	LEU-3			TYR-23	TRP-24
	PRO-318	LEU-3				
	TRP-30	PRO-4				
	MET-314	PRO-4				
	MET-314	VAL-5				
	PRO-318	VAL-5				
	TYR-23	TRP-24				
	TYR-23	PRO-25				
	LEU-27	PRO-25				
	LEU-33	PRO-25				
	LEU-27	VAL-26				
	TRP-30	VAL-26				
Varv peptide E	MET-314	PRO-17			TYR-23	TRP-23
	TYR-23	TRP-23				
	LEU-27	TRP-23				
Palicourein	PRO-271	VAL-9	ASP-274	ARG-8		
	VAL-346	TYR-15	ASP-349	ARG-8		
			LYS-423	ASP-28		
			GLU-132	LYS-32		
			GLU-127	ARG-33		
Vhl-1	ALA-28	PHE-12				
	MET-314	ILE-30				
	PRO-318	ILE-30				
Griffithsin	VAL-154	LEU-46	ASP-453	ARG-145		
	PRO-117	PRO-48	ASP453	HIS-159		
	TRP-145	PRO-48				
	PRO-117	TYR-85				
	PRO-120	TYR-85				
Cycloviolacin VY1	TRP-30	PRO-9	ASP-337	LYS-22		
	TYR-23	ILE-21	GLU-39	LYS-26		
	LEU-27	ILE-21				

Interactions: * within 5 A˚; # within 6 A˚; § within 4.5 and 7 A˚. AR= ACE2 residues, PR=Peptide residues

Table 5: ACE2-peptide interactions involving hydrogen bonds

	Main Chain-Main Chain Hydrogen Bonds		Main Chain-Side Chain Hydrogen Bonds		Side Chain-Side Chain Hydrogen Bonds	
	AR	PR	AR	PR	AR	PR
Circulin A			TYR-23	LEU-15	ASP-337	SER-18
			GLU-311	ASN-27	ASN-31	CYS-19
			TYR-23	ALA-14	GLU-311	ASN-27
			TYR-23	LEU-15	ASN-31	CYS-19
			TYR-23	CYS-17		
			ASN-31	CYS-19		
			ASN-43	CYS-5		
			ASN-43	VAL-6		
			ASN-43	TRP-7		
Kalata B1			TRP-30	PRO-4	ASN-40	GLU-8
			THR-34	PRO-4	ASN-31	THR-9
			ASN-35	CYS-6	ASN-312	SER-23
			ASN-35	GLY-7		
			ASN-312	TRP-24		
			GLN-322	VAL-5		
Varv peptide E			TYR-23	THR-13	ASN-312	THR-13
			ASN-312	ASN-15	ASN-312	ASN-15
			THR-34	GLY-18	ASN-31	SER-20
			ASN-31	CYS-19	ASN-43	ASN-29
			ASN-31	SER-20		
			GLU-39	ASN-29		
			ASN-40	ASN-29		
			TYR-23	GLY-12		
Palicourein			THR-344	TYR-15	ASP-349	THR-6
			SER-262	ARG-26	ASN-272	ARG-8
			SER-262	ASP-28	ASP-349	ARG-8
			ASP-274	THR-6	ASN-136	LYS-32
			LYS-345	CYS-3	THR-276	GLU-5
					LYS-345	CYS-3
					THR-347	GLU-5
Vhl-1			TRP-30	CYS-1	ASN-40	THR-13
			TRP-30	GLY-2	GLU-311	ASN-22
			ASN-31	CYS-1	GLN-307	LYS-22
			THR-34	CYS-1	GLU-311	TYR-26
			GLN-322	LEU-27	ASN-40	THR-13

			ARG-339	GLY-2	GLU-311	ASN-22
			GLU-311	TYR-26		
			SER-313	SER-31		
Griffithsin	CYS-115	LEU-46	PRO-120	ASN-45	CYS-123	ASN-45
			GLN-121	ASN-45	SER-152	THR-49
			CYS-115	LEU-46	GLU-153	THR-49
			SER-152	THR-49	TYR-479	THR-51
			GLU-153	THR-49	ASP-453	ARG-145
Cycloviolacin VY1	ALA-28	CYS-19	ASN-312	ILE-8	ASN-43	SER-18
			ASN-35	VAL-14	ASN-31	CYS-19
			ASN-31	CYS-19	ASN-31	CYS-19
			ASN-31	CYS-19	TYR-23	LYS-22
			ASN	GLY-28	ASP-337	LYS-22

Molecular dynamics simulations

The ACE2-peptide interactions were further evaluated via molecular dynamics simulations analysis to examine the fluctuation of the respective amino acids in the individual complex and their respective conformational stability (Jamroz *et al*. 2013). Ten (10) different models were predicted by the exploratory simulation per run and the first model of each was selected based on its best structural heterogeneity, and stability. Significant changes were noticed in the structural flexibility of ACE2 investigated based on the root mean square fluctuation (RMSF). This is possibly due to the interaction of the peptides with the interface of the receptor when compared to the wild-type (Figure 3). The contact map presents the interaction interface between all the atoms in the wild-type ACE2 and the complexes. All the peptides, except Vhl-1 and varv peptide E showed residues with comparatively higher RMSF values than the wild-ACE2, thus indicating the binding of circulin A, kalata B1, palicourein, griffithsin, and cycloviolacin VY1could induce flexibility in further ACE2. The associated lower RMSF values computed from the bindings of Vhl-1 and varv peptide E with ACE2 could suggest induced limited fluctuation of complexes in the course of the simulation process. Thus, the binding of circulin A, kalata B1, palicourein, griffithsin, and cycloviolacin VY1 could influence the reliability of ACE2 secondary structure and subsequently the functional properties of the receptor and its innate capability to bind the coronavirus spike protein. This observation could substantiate use of peptides as better therapeutic agents over small molecule-based on their high target specificity and potency (Zompra *et al*., 2009; Vlieghe *et al*., 2010; Craik *et al*., 2013; Holohan *et al*., 2013).

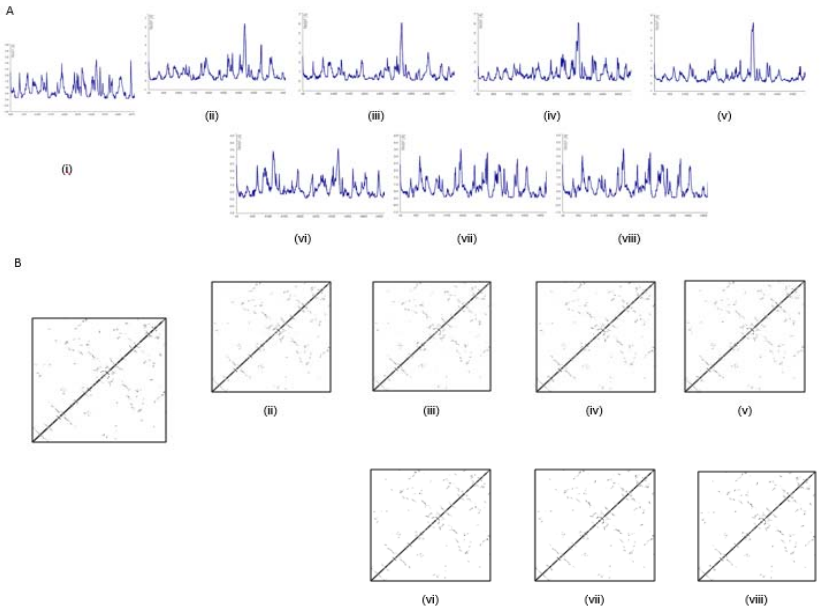

Figure 3: Molecular dynamic simulations representing (A) Fluctuation plot and (B) Contact map of (i) Wild-type ACE2 and complex of ACE2 with (ii)Circulin A, (iii) Kalata B1, (iv) Varv peptide E, (v) Palicourein, (vi) Vhl-1, (vii) Griffithsin, (viii) Cycloviolacin VY1

Conclusion

The peptides considered in this study especially Kalata B1, Vhl-1, and Cycloviolacin VY1 as well as Varv peptide E have the abilities to modulate ACE2 which might be due to their high cyclic content compared to others. These peptides could play significant roles as modulators of ACE2 and could be repositioned as anti-viral agents to prevent the entry and further replication of SARS-CoV-2. However, synthesis and standardization of the selected peptides with their corresponding experimental evaluations are recommended further studies.

Acknowledgements

The support provided by the staff of Department Biological Sciences, College of Natural and Applied Sciences, McPherson University, Seriki Sotayo, Ogun State, Nigeria is well appreciated.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

REFERENCES

1. Akkam, Y. A Review of Antifungal Peptides: Basis to New Era of Antifungal Drugs. Jordan Journal of Pharmaceutical Sciences 2016; 9(1): 51-75.
2. Al-Shar'i, N.A., Hassan, M.A., Al-Barqi, H.M., Al-Balas, Q.A. and El-Elimat, T. Discovery of Novel Glyoxalase-I Inhibitors Using Computational Fragment-Based Drug Design Approach. Jordan Journal of Pharmaceutical Sciences 2020; 13(2): 225-245.
3. Artimo P., Jonnalagedda M., Arnold K., Baratin D., Csardi G., de Castro E., Duvaud S., Flegel V., Fortier A., Gasteiger E., Grosdidier A., Hernandez C., Ioannidis V., Kuznetsov D., Liechti R., Moretti S., Mostaguir K., Redaschi N., Rossier G., Xenarios I. and Stockinger H. ExPASy: SIB bioinformatics resource portal. *Nucleic Acids Res.* 2012; 40(1): 597-603
4. Chang K.Y. and Yang J.R. Analysis and Prediction of

Highly Effective Antiviral Peptides Based on Random Forests. *PLOS ONE* 2013; 8(8): e70166. https://doi.org/10.1371/journal.pone.0070166

5. Chen F., Ma B., Yang Z.C., Lin G. and Yang D. Extraordinary metabolic stability of peptides containing alpha-aminoxy acids. *Amino Acids* 2011; 43(1): 499-503
6. Craik D.J., Fairlie D.P., Liras S. and Price D. The future of peptide-based drugs. *Chem Biol Drug Des*. 2013; 81: 136–147, doi: 10.1111/ cbdd.12055
7. Crowell J.A. The chemopreventive agent development research programming the Division of Cancer Prevention of the US National Cancer Institute: An overview. *Eur. J. Cancer* 2005; 41: 1889 -1910
8. European Center for Disease control and Prevention (2020). Covid-19 Situation update worldwide. https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases. Accessed 08 May, 2020
9. Feng T., Chen F., Kang Y., Sun H.Y., Liu H., Li D., Zhu F. and Hou T.J. HawkRank: a new scoring function for protein-protein docking based on weighted energy terms. *J. Cheminformatics* 2017*;* 9(1):66.
10. Hashimoto T., Perlot T., Rehman A., Trichereau J., Ishiguro H., Paolino M., Sigl V., Hanada T., Hanada R., Lipinski S., Wild B., Camargo S.M., Singer D., Richter A., Kuba K., Fukamizu A., Schreiber S., Clevers H., Verrey F., Rosenstiel P. and Penninger J.M. ACE2 links amino acid malnutrition to microbial ecology andf intestinal inflammation. *Nature* 2012; 487 (7408):477–481.
11. Holohan C., Van Schaeybroeck S., Longley D.B. and Johnston P.G. Cancer drug resistance: an evolving paradigm. Nat Rev Cancer 2013; 13: 714–726. doi: 10.1038/nrc3599
12. Huang C., Wang Y., Li X., Ren L., Zhao J., Hu Y., Zhang L., Fan G., Xu J., Gu X., Cheng Z., Yu T., Xia J., Wei Y., Wu W., Xie X., Yin W., Li H., Liu M., Xiao Y., Gao H., Guo L., Xie J., Wang G., Jiang R., Gao Z., Jin Q., Wang J. and Cao B. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 2020;. https ://doi.org/10.1016/S0140 -6736(20)30183 -5
13. Jamroz M., Kolinski A. and Kmiecik S. CABS-fex: server for fast simulation of protein structure
14. fuctuations. *Nucleic Acids Res.* 2013; 41: W427-W431.
15. Jia H. (2016). Pulmonary Angiotensin-Converting Enzyme 2 (ACE2) and Inflammatory Lung Disease. Shock. *Augusta, Ga*; 46 (3): 239–48.
16. Kuriata A., Gierut A.M., Oleniecki T., Ciemny M.P., Kolinski A., Kurcinski M. and Kmiecik S. CABS-flex 2.0: a web server for fast simulations of flexibility of protein structures. *Nucleic Acids Research* 2018; 46(W1): W338-W343. doi: 10.1093/nar/gky356.
17. Lu L., Liu Q., Zhu Y., Chan K.H., Qin L., Li Y., Wang Q., Chan J.F., Du L., Yu F., Ma C., Ye S., Yuen K.Y., Zhang R. and Jiang S. Structure-based discovery of Middle East respiratory syndrome coronavirus fusion inhibitor. *Nature Communications* 2014; 5:3067
18. Mathur D., Singh S., Mehta A., Agrawal P. and Raghava G.P.S. In silico approaches for predicting the half-life of natural and modified peptides in blood. *PLOS ONE* 2018; 13(6): e0196829. https://doi.org/10.1371/ journal. pone.0196829
19. Mehta D., Anand P., Kumar V., Joshi A., Mathur D., Singh S., Tuknait A., Chaudhary K., Gautam S.K., Gautam A., Varshney G.C. and Raghava G.P.S. ParaPep: a web resource for experimentally validated antiparasitic peptide sequences and their structures. Database (Oxford). 2014; 2014: bau051±bau051. https://doi.org/10.1093/ database/ bau051
20. Mustafa S., Balkhy H. and Gabere M. Peptide-Protein Interaction Studies of Antimicrobial Peptides Targeting Middle East Respiratory Syndrome Coronavirus Spike Protein: An In Silico Approach. Advances in Bioinformatics Volume 2019, Article ID 6815105, 16 pages https://doi.org/10.1155/2019/6815105
21. Pontius J., Richelle J. and Wodak S.J. Deviations From Standard Atomic Volumes as a Quality Measure for Protein Crystal Structures. *Journal of Molecular Biology.* 1996; 264(1): 121-36. doi: 10.1006/jmbi.1996.0628.
22. Roxin A. and Zheng, G. Flexible or fixed: A comparative review of linear and cyclic cancer-targeting peptides. *Future*

Med. Chem. 2012; 4(12): 1601-1618. doi: 10.4155/fmc.12.75

23. Suenaga A., Okimoto N., Hirano Y. and Fukui K. (2012). An efficient computational method for calculating ligand binding affinities. *PLOS One* 2012; 7: e42846. doi: 10.1371/journal.pone.0042846
24. Tikellis C. and Thomas M.C. Angiotensin-Converting Enzyme 2 (ACE2) Is a Key Modulator of the Renin Angiotensin System in Health and Disease. *International Journal of Peptides* 2012; 256294:1-8 doi:10.1155/2012/256294.
25. Tina K.G., Bhadra R. and Srinivasan N. PIC: protein interactions calculator. *Nucleic Acids Research* 2007; 35(2):. W473-W476.
26. Vlieghe P., Lisowski V., Martinez J. and Khrestchatisky M. Synthetic therapeutic peptides: Science and market. *Drug Discovery. Today* 2010; 15(1-2): 40-56
27. Wang G., Li X. and Wang Z. APD3: the antimicrobial peptide database as a tool for research and education. Nucleic Acids Research 2016; 44(D1): .D1087-D1093,2016.
28. Wan Y., Shang J., Graham R., Baric R.S. and, Li F. Receptor recognition by novel coronavirus from Wuhan: an analysis based on decade-long structural studies of SARS. *Journal of Virology* 2020 https ://doi.org/ 10.1128/jvi.00127-20
29. Weng G.Q., Wang E.C., Wang Z., Liu H., Li D., Zhu F. and Hou T.J. HawkDock: a web server to predict and analyze the structures of protein-protein complexes based on computational docking and MM/GBSA. *Nucleic Acids Research* 2019; 47(W1): W322-W330.
30. Wu K.L., Peng G.Q., Wilken M., Geraghty R.J. and Li F. Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. *Journal of Biological Chemistry* 2012; 287: 8904–8911
31. Xia S., Yan L., Xu W., Agrawal A.S., Algaissi A., Tseng C.K., Wang Q., Du L., Tan W., Wilson I.A., Jiang S., Yang B. and Lu L. A pan-coronavirus fusion inhibitor targeting the HR1 domain of human coronavirus spike. *Science Advances* 2019; 5: 4580.
32. Xia S., Zhu Y., Liu M., Lan Q., Xu W., Wu Y., Ying T., Liu S., Shi Z., Jiang S. and Lu L Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. *Cellular & Molecular Immunology* 2020. https://doi.org/10.1038/s41423-020-0374-2.
33. Xu X., Chen P., Wang J., Feng J., Zhou H., Li X., Zhong W. and Hao P. Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its Spike protein for risk of human transmission. *Science China Life Sciences* 2020. https ://doi.org/10.1007/s1142 7-020-1637-5
34. Yan R., Zhang Y., Li Y., Xia L., Guo Y. and Zhou Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. *Science* 2020; 367 (6485): 1444-1448
35. Yang J. and Zhang Y. I-TASSER server: new development for protein structure and function predictions. *Nucleic Acids Research* 2015; 43: W174-W181, 2015
36. Yu L., Yuan K., Phuong H.T., Park B.M. and Kim S.H. Angiotensin-(1-5), an active mediator of renin-angiotensin system, stimulates ANP secretion via Mas receptor. *Peptides* 2016; 86: 33-41
37. Zhang C., Freddolino P.L. and Zhang Y. COFACTOR: improved protein function prediction by combining structure, sequence and protein–protein interaction information. *Nucleic Acids Research* 2017; 45: W291-W299.
38. Zhang H., Penninger J.M., Li Y., Zhong N. and Slutsky A.S. Angiotensin-converting enzyme 2 (ACE2) as a SARS-CoV-2 receptor: molecular mechanisms and potential therapeutic target. *Intensive Care Medicine* 2020. https://doi.org/10.1007/s00134-020-05985-9.
39. Zheng Y.Y., Ma Y.T., Zhang J.Y. and Xie X. COVID-19 and the cardiovascular system. *Nature Reviews Cardiology* 2020. https://doi.org/10.1038/s41569-020-0360-5.
40. Zompra A.A., Galanis A.S., Werbitzky O. and Albericio F. Manufacturing peptides as active pharmaceutical ingredients. *Future Med Chem* 2009; 1: 361-77.

الدراسات النظرية للببتيدات ذات الأصل النباتي التي تستهدف الإنزيم المحول للأنجيوتنسين البشري المرتبط بالإنزيم carboxypeptidase

باباتوندي جوزيف أوسو[1] وإيج فرانسيس أولوي[2] وأديبيجو أبيرواغبا[3]

1 قسم الكيمياء الحيوية ، جامعة ماكفرسون ، سيريكي سوتايو ، ولاية أوجون ، نيجيريا

2 كلية الصيدلة وعلوم الجزيئات الحيوية ، جامعة ليفربول جون مورس ، ليفربول

3. قسم الكيمياء الحيوية ، جامعة إيلورين ، إيلورين ، نيجيريا

ملخص

خلفية

إن قابلية تعرض الرئتين والأمعاء والقلب والكلى لغزو **SARS-CoV-2** تعتمد على التعبير العالي عن إنزيم **carboxypeptidase** المحول للأنجيوتنسين **(ACE2)** على السطح الخارجي للخلايا في هذه الأعضاء .يؤكد هذا الوضع الواضح للتفاعل بين بروتينات ارتفاع **SARS-CoV-2** و **ACE2** على أهمية مستقبلات **ACE2** في انتشار فيروسات كورونا .بحثت هذه الدراسة في إمكانات الارتباط لبعض الببتيدات النباتية المختارة **(Circulin A ، kalata B1 ، Varv peptide E ، Palicourein ، Vhl-1 ، griffithsin ، cycloviolacin VY1)** إلى **ACE2** كنهج تنبؤي في منع غزو **.SARS-CoV-2**

أساليب

تم استرداد الببتيدات من قاعدة بيانات الببتيد المضاد للميكروبات وتم التنبؤ بخصائصها الفيزيائية والكيميائية باستخدام أداة **.ProtParam** تم حساب وضع الربط والطاقات الحرة الملزمة من خلال خوادم **HawkDock** بينما تم تقييم المرونة الهيكلية واستقرار مجمعات **ACE2-peptide** عبر خادم**.CABS-flex 2.0**

نتائج

لوحظ أن درجات الربط للببتيدات تجاه **ACE2** أظهرت ارتباطات ارتباط جيدة مع **griffithsin** الذي حصل على أفضل درجة ربط من خلال رتبة **Hawkdock** بينما حصلت **kalata B1** على أقل درجة ربط .أظهر تحليل الميكانيكا الجزيئية / منطقة سطح الولادة المعممة أن الطاقة الحرة الملزمة تتراوح **39.99-** و **3.96-** كيلو كالوري / مول مع **Vhl-1** الذي يحتوي على أعلى طاقة حرة و **palicourein**الذي يحتوي على أقل طاقة حرة

الاستنتاجات

تشير نتائج الدراسة إلى أن الببتيدات النباتية المختارة وخاصة **kalata B1** و **vhl-1** و **cycloviolacin VY1** يمكن أن تكون مُعدِّلات واعدة لـ **ACE2** وتمنع ارتباط المجال **S1** لبروتين **SARS-CoV-2 S** وما يترتب على ذلك من خلايا خلوية .دخول**.SARS-CoV-2**

الكلمات الدالة: الببتيدات ، الإنزيم المحول للأنجيوتنسين 2 ، ألفة ملزمة ، إمكانية التعديل ، سارس-كوف.

تاريخ استلام البحث 2020/6/9 وتاريخ قبوله للنشر 2021/4/17.