NATURAL PEPTIDES VERSUS COVID-19: INFORMATION FOR CONSIDERATION

Oleg Kit[1], Yuriy Kit[2*]

1- *Lviv Institute of the Interregional Academy of Personnel Management, Het'man Mazepa St., 7900, Lviv, Ukraine*
2- *Institute of Cell Biology National Academy of Science of Ukraine, Drahomanov St., 14/16, Lviv, 79005, Ukraine*

**Correspondence to: kit@cellbiol.lviv.ua*

ABSTRACT

Coronavirus disease 19 (COVID-19) is an emerging global health crisis, where SARS-CoV-2 is the novel coronavirus responsible for this disease. The virus enters into human cells by binding to angiotensin-converting enzyme 2 (ACE2) via the receptor binding domain of its spike protein. Disrupting the SARS-CoV-2-RBD binding to ACE2 with designer drugs has the potential to inhibit the virus from entering human cells, presenting a new modality for therapeutic intervention. Recently, Zhang et al., using molecular dynamics simulations demonstrated that the ACE2 peptidase domain α1 helix is important for binding SARS-CoV-2-RBD. The 23-mer synthetic peptide fragment of the ACE2 α1 helix is capable of effectively blocks the SARS-CoV-2 spike protein interaction with ACE2. We suggested, that numerous well known natural peptides are a source for searching the new agents for blocking SARS-CoV-2 – ACE2 interaction. 14 natural peptides that are present or may be present in human fluids (blood plasma, saliva, etc.), were taken us for further investigation using accessed bioinformatics services. Our data show, that only four (angiotensins I and II, bradykinin and beta casokinin 10) from the studied natural peptides, possess high affinity with SARS-CoV-2 spike protein. We suggest that these peptides are serve as potential platform for development of novel drags for treatment of COVID-19 disease.

Key words : SARS-CoV-2-RBD, natural peptide binder, protein-protein interaction inhibitor, protein-protein docking

INTRODUCTION

Coronavirus disease (COVID-19) is an emerging global health crisis [1, 2]. SARS-CoV-2 is the novel coronavirus responsible for this disease [3]. It initiates entry into human cells by binding to angiotensin-converting enzyme 2 (ACE2) via the receptor binding domain (RBD) of its spike protein (S) [4]. Disrupting the SARS-CoV-2-RBD binding to ACE2 with designer drugs has the potential to inhibit the virus from entering human cells, presenting a new modality for therapeutic intervention [5]. Recently, using molecular dynamics simulations based on the solved ACE2 and SARS-CoV-2-RBD co-crystal structure, it has been demonstrated that the ACE2 peptidase domain (PD) α1 helix is important for binding SARS-CoV-2-RBD [6]. The 23-mer synthetic

peptide fragment of the ACE2 α1 helix is capable of effectively blocks the SARS-CoV-2 spike protein interaction with ACE2. This peptide provides new avenues for COVID-19 treatment and diagnostic modalities by blocking the SARS-CoV-2 spike protein interaction with ACE2 [7]. This work provoked us to suggest that numerous well known artificial and natural peptides can serve as potential agents for COVID-19 treatment. 14 natural peptides, that are present or may be present in human fluids (blood plasma, saliva, etc.), were taken us for further investigation using accessed bioinformatics services.

METHODS

The structures of peptides were assessed by PEP-FOLD3 SERVER (Université Paris Diderot, Sorbonne Paris Cité, Paris, France) (https://mobyle.rpbs.univ-paris-diderot.fr/cgi-bin/portal.py#forms::PEP-FOLD3) [8]. RBD protein structure was obtained using I-TASSER (Medical School, University of Michigan, ANN Arbor MI, USA) (https://zhanglab.ccmb.med.umich.edu/I-TASSER/) [9]. Spike structure was taken from COVID-19 Docking Server (Institute of Bioinformatics and Medical Engineering, School of Electrical and Information Engineering, Jiangsu University of Technology,Changzhou, China) (https://ncov.schanglab.org.cn/repncov/S_trimer.pdb) [10]. This structures were saved in PDB format and used for docking. Docking of peptides with RBD and Spike were performed using Cluspro 2.0 Server (Vajda group, Boston University, Boston MA, USA) (https://cluspro.bu.edu/peptide/index.php) [11] and HDOCK Server (Lab of Bioinformatics and Molecular Modeling, School of Physics, Huazhong University of Science and Technology, Wuhan, Hubei, China) (http://hdock.phys.hust.edu.cn/) [12]. Binding affinity (ΔG) and dissociation constant (Kd) were predicted on the PRODIGY server (Computational Structural Biology group, Utrecht University, Utrecht, Netherlands) (https://bianca.science.uu.nl/prodigy/) [13].

RESULTS AND DISCUSSION

Recently have been published data demonstrated that a 23-mer peptide (sequence selected from the ACE2 α1 helix sequence (IEEQAKTFLDKFNHEAEDLFYQS) can

serve as a SARS-CoV-2 spike protein binder [7]. We used this sequence as "gold" standard to estimate the level of affinity of natural peptides toward spike virus protein as well as its receptor-binding domain. To study, we basically used the sequences of peptides, that engage in the renin-angiotensin system, where ACE2 plays a crucial role.

As a stronghold of ligand-receptor affinity we chased the constant dissociation value in ratio < E-07 M. The summarizing data of computing simulating is demonstrated on Table 1. It was shown, that 3 and 4-mer natural peptides of different origination possesses low affinity to whole-lengths spike virus's protein as well as its RBD domain. Among 8-mer and 10-mer peptides were observed preferentially high affinity to a whole spike virus protein, meanwhile, for two of them was shown also high affinity to its RBD domain. Fig.1 illustrates 3-D images of interaction of detected by us RBD-binder's peptides with RBD. Interestingly, that all detected peptide with high affinity to RBD preferentially locate in the residues responsible for interaction of SARS-CoV-2 spike protein with ACE2 receptor on epithelial cells. Characteristics of most attracted peptides are established below. **Angiotensins** are peptids that causes vasoconstriction and an increase in blood pressure. It is part of the renin–angiotensin system, which regulates blood pressure. Angiotensin also stimulates the release of aldosterone from the adrenal cortex to promote sodium retention by the kidneys [18]. **Bradykinin** is an inflammatory mediator. It is a peptide that causes arterioles to dilate (enlarge) via the release of prostacyclin, nitric oxide, and Endothelium-Derived Hyperpolarizing Factor, it makes the veins constrict, via prostaglandin F2, and thereby lead to leakage into capillary beds, due to the increased pressure in the capillaries [19]. A class of drugs called angiotensin converting enzyme inhibitors (ACE inhibitors) increase bradykinin levels by inhibiting its degradation, thereby increasing its blood pressure lowering effect. ACE inhibitors are FDA approved for the treatment of hypertension and heart failure. **Beta-casokinin 10** is bioactive component of milk and dairy products [14,15].

Conclusion. Obtained data demonstrate that cohort of natural peptides of different origination contains ligands with high affinity to SARS-CoV-2 spike protein. We suggest that this approach allows to find peptides, that can be used as a perspective platform in order to development of novel drags for treatment of COVID-19 disease.

REFERENCES

1. Wu, F. et al. 2020. A new coronavirus associated with human respiratory disease in China. Nature. 579, 265–269. doi:10.1038/s41586-020-2008-3
2. Wang, C., Horby, P. W., Hayden, F. G. & Gao, G. F. A novel coronavirus outbreak of global health concern. The Lancet. 2020. 395: 470–473. doi: 10.1016/S0140-6736(20)30185-9
3. Walls A.C, Park Y.J., Tortorici M.A., Wall A., McGuire A.T. Veesler D. 2020. Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell. 2020. S0092-8674(20)30262-2. doi: 10.1016/j.cell.2020.02.058.
4. Yan R,. Zhang Y, Li Y., Xia L., Guo Y., Zhou Q., 2020. Structural basis for the recognition of the SARS-286 CoV-2 by full-length human ACE2. Science. 2020 Mar 27;367(6485):1444-1448. doi: 10.1126/science.abb2762.
5. Wan Y., Shang J.,. Graham R, Baric R.S., Li F. 2020. Receptor recognition by novel coronavirus from Wuhan: An analysis based on decade-long structural studies of SARS. J Virol, J Virol. 94(7). . doi: 10.1128/JVI.00127-20
6. M. Hoffmann, H. Kleine-Weber, S. Schroeder, N. Kruger, T. Herrler, S. Erichsen, T.S. Schiergens, G.Herrler, N.H. Wu, A. Nitsche, M.A. Muller, C. Drosten, and S. Pohlmann, SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and is Blocked by a Clinically Proven Protease Inhibitor. Cell, 2020. 280. doi: 10.1016/j.cell.2020.02.052
7. Zhang G., Pomplun S., Loftis A. R., Loas A., Pentelute B. L. The first-in-class peptide binder to the SARS-CoV-2 spike protein. bioRxiv preprint. doi: 10.1101/2020.03.19.999318
8. Lamiable A, Thévenet P, Rey J, Vavrusa M, Derreumaux P, Tufféry P. PEP-FOLD3: faster de novo structure prediction for linear peptides in solution and in complex. Nucleic Acids Res. 2016 Jul 8;44(W1):W449-54. doi: 10.1093/nar/gkw329

9. J Yang, Y Zhang. I-TASSER server: new development for protein structure and function predictions. Nucleic Acids Research, 43: W174-W181 (2015). doi: 10.1093/nar/gkv342
10. Kong R, Yang G B, Xue R, Liu M, Wang F, Hu J P, Guo X Q, Chang S. COVID-19 Docking Server: An interactive server for docking small molecules, peptides and antibodies against potential targets of COVID-19. 2020. arXiv:2003.00163v1 [q-bio.BM].
11. Kozakov, Dima et al. "The ClusPro web server for protein-protein docking." Nature protocols vol. 12,2 (2017): 255-278. doi:10.1038/nprot.2016.169
12. Yan Y, Zhang D, Zhou P, Li B, Huang S-Y. HDOCK: a web server for protein-protein and protein-DNA/RNA docking based on a hybrid strategy. Nucleic Acids Research, 45: W365-373, 2017. doi: 10.1093/nar/gkx407
13. Xue L., Rodrigues J., Kastritis P., Bonvin A.M.J.J., Vangone A., "PRODIGY: a web-server for predicting the binding affinity in protein-protein complexes", Bioinformatics, doi: 10.1093/bioinformatics/btw514 (2016).
14. Pepe G, Tenore G.C., Mastrocinque R., Stusio P., Campiglia P. Potential Anticarcinogenic Peptides from Bovine Milk. J. Amino Acids. 2013; 2013:939804. doi:10.1155/2013/939804.
15. Ledesma-Martínez E, Aguíñiga-Sánchez I, Weiss-Steider B, Rivera-Martínez AR, Santiago-Osorio E. Casein and Peptides Derived from Casein as Antileukaemic Agents.J Oncol. 2019 8;2019:8150967. doi: 10.1155/2019/8150967
16. Myronovkij S, Negrych N, Nehrych T, Tkachenko V, Souchelnytskyi S, Stoika R, Kit Y. Identification of SER-PRO-CYS Peptide in blood serum of multiple sclerosis patients. Protein Pept Lett. 2016;23(9):808-11. doi:10.2174/0929866523666160622215628
17. Young Woo Park, Myoung Soo Nam. Korean J. Food Sci. An. 35 (6): 831~840(2015) Korean Society for Food Science of Animal Recources. doi: 10.5851/kosfa.2015.35.6.831

18. M. Ruiz-Ortega, O. Lorenzo, M. Rupérez, V. Esteban, Y. Suzuki, S. Mezzano, J.J. Plaza, J. Egido. Role of the Renin-Angiotensin System in Vascular Diseases Expanding the Field Hypertension. 2001;38:1382-1387. doi: 10.1161/hy1201.100589.
19. Hornig B., Kohler C., Drexler H. Role of Bradykinin in Mediating Vascular Effects of Angiotensin-Converting Enzyme Inhibitors in Humans. Circulation. Volume 95, Issue 5, 4 March 1997, Pages 1115-1118/ doi: 10.1161/01.CIR.95.5.1115

Table 1. Summarized data the peptides affinity to the spike SARS-CoV-2 protein and its RBD domain

No	Peptide	Sequence	HDOCK Docking Score (Spike)	HDOCK Docking Score (RBD)	ClusPro (PRODIGY) (Spike)		ClusPro (PRODIGY) (RBD)		Ref
					ΔG (kcal/ mol)	Kd (M)	ΔG (kcal/ mol)	Kd (M)	
1	casokinin 1	IPP	-144.53	-97.31	-5.9	4.8E-05	-7.9	1.6E-06	14,15
2	casokinin 2	VPP	-137.22	-94.07	-7.6	2.9E-06	-7.9	1.6E-06	14,15
3	prosercyn	SPC	-124.16	-93.42	-8.5	5.4E-07	-7.9	1.7E-06	16
4	opioid peptide	YGGF	-191.23	-161.28	-6.8	9.6E-06	-8.9	3.1E-07	15,17
6	2(prosercyn)	SPCCPS	-149.06	-115.74	-9.5	1.0E-07	-8.3	8.2E-07	16
7	alpha - casomorphin 6	RYLGYL	-206.57	-182.12	-9.5	1.1E-07	-7.2	4.8E-06	15,17
8	alfa-casomorphin 7	RYLGYLE	-197.93	-182.17	-10.7	1.4E-08	-8.3	8.0E-07	15,17
9	Beta-casomorphin 7	YPFPGPI	-206.07	-150.31	-8.8	3.5E-07	-7.9	1.5E-06	15
10	Angiotensin II	DRVYIHPF	-237.65	-180.80	-10.8	1.1E-08	-9.3	1.5E-07	18
11	Bradykinin	RPPGFSPFR	-228.50	-185.08	-9.0	2.5E-07	-9.9	5.8E-08	18
12	Angiotensin I	DRVYIHPFHL	-250.26	-202.96	-12.5	6.9E-10	-9.3	1.5E-07	19
13	Beta-casokinin 10	YQQPVLGPVR	-214.68	-169.06	-10.9	9.5E-09	-9.7	7.7E-08	14,15
14	RBP-binding peptide	IEEQAKTFLDKF NHEAEDLFYQS	-240.53	-230.76	-11.8	2.1E-09	-11.9	2.0E-09	7

Standard SARS-CoV-2-RBD peptide binder is marked with yellow. Peptides with highest affinity to SARS-CoV-2-Spark protein and its RBD are marked with blue.

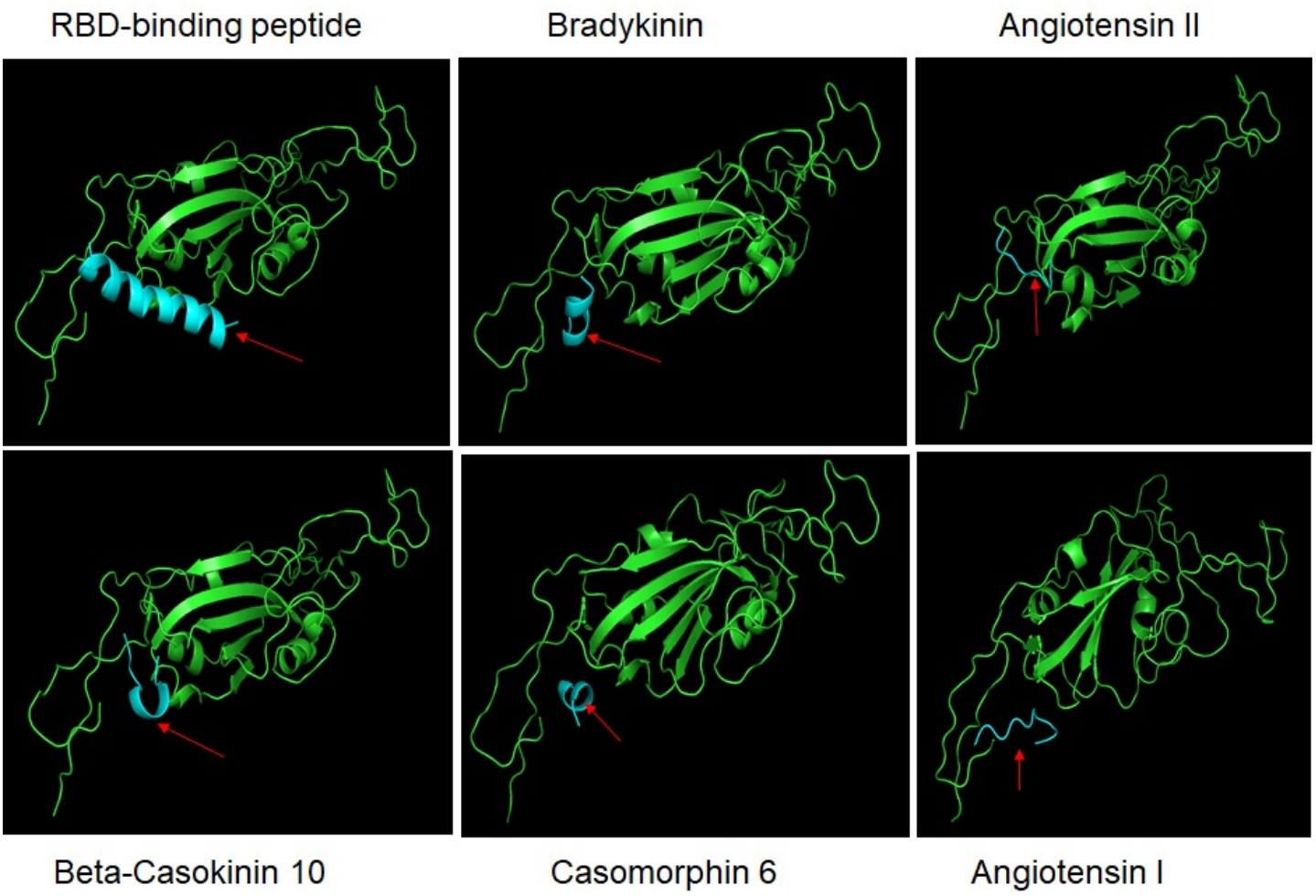

Fig 1. 3D-dimensional images of structure the RBP-peptide's ligand complexes. Red arrows are pointed position of the peptide ligands.