Original Article

Restraint of VP1 Protein of Foot and Mouth Disease Virus using Specific Antiviral Peptides: an *in Silico* Investigation

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ABSTRACT

Foot and mouth diseases are among the important threats in the animal husbandry industry which lead to huge economic losses. In this regard, the current project aimed to inhibit the VP1 protein of foot and mouth disease viruses using specific peptides. For this purpose, a wide range of potential antiviral peptides were collected from the database. Physicochemical properties, hydrophobicity/hydrophilicity, and solubility properties of potential antiviral peptides were investigated using reliable servers. Afterward, the tertiary structures of the selected peptides along with the VP1 protein were modeled by the I-TASSER server. Moreover, interactions between VP1 protein and selected antiviral peptides were investigated using the ClusPro 2.0 server. Finally, the outputs of molecular docking were assessed by LigPlot+ and visualized by PyMol software. The results revealed that *Dermaseptin-3*, *Ginkbilobin*, *Circulin-F*, *Maximin1*, *Cycloviolin-A*, *Cycloviolin-D*, *Circulin-C*, *Cycloviolin-C*, and *Antihypertensive protein BDS-1* peptides with a hydrophobicity value of > 30 were soluble with positive instability index and positive net charge. Moreover, the results of the molecular docking process demonstrated that *Dermaseptin-3* and *Ginkbilobin* peptides could strongly inhibit the VP1 protein using 10 hydrogen bonds. Therefore, these two peptides, which had the most hydrogen bonds, were introduced as the best anti-foot and mouth disease virus peptides to apply.

Keywords: Antimicrobial Peptide, Foot and mouth disease, In Silico, VP1

1. Introduction

Foot and mouth disease (FMD) is caused by a virus (FMD virus) that contains positive-strand ribonucleic acid (RNA) and belongs to the genus *Aphthovirus* and *Picornaviridae* family (1). The FMD has highly variable RNA that engenders O, A, C, Asia 1, SAT 1, SAT 2, and SAT 3 serotypes and a wide range of subtypes. Regarding the distribution of seven FMD virus serotypes worldwide, serotypes A and O have the highest distribution rates. They are the most antigenically variable European-Asian serotypes and are also important serotypes in the Middle East and especially in Iran (2). The FMD virus contains an icosahedral protein capsid (containing 20 equilateral triangles) and an RNA genome that encodes a polyprotein with four structural proteins (including VP1, VP2, VP3, and VP4) and eight non-structural proteins (including L, 2A, 2B, 2C, 3A, 3B, 3C, and 3D polymerase) (3, 4).

The capsid of the FMD virus consists of 60 structural units called protomers, and each protomer consists of 1AB, 1C, and 1D proteins (5). The X-ray crystallography of the VP1 protein has confirmed that the tertiary structure of this protein is in the beta-sheet form which can create some loops on the surface of the virus. In general, the G-H loop with highly variable regions is known as the most important loop of VP1 protein, including 140-160 residues of VP1 protein. In fact, the flexibility of the G-H loop plays a key role in the binding and entry of the virus into the host cell. It has been reported that the binding and entry of the virus into the host cell completely decreases while the G-H loop is located on the viral cover due to the deformation of disulfide bonds (6, 7). The G-H loop has a protected region containing arginine (Arg), glycine (Gly), and aspartic acid (Asp) amino acids which are located in 145, 146, and 147 positions, respectively. These amino acids play a vital role in binding the virus to the host cell receptor (8).

Antimicrobial peptides (AMPs) are small biological molecules that play an important role in the defense mechanisms of organisms by invading microorganisms, maintaining gastrointestinal homeostasis, and modulating inflammatory responses. These compounds are found in both eukaryotes and prokaryotes which exhibit a wide variety of antimicrobial activities (antibacterial, antiviral, and antifungal) (9). In general, these peptides are divided into two categories in terms of charge, namely cationic and anionic.

Moreover, they are divided into four groups in terms of their secondary structure, including α-helix peptides, β-plates, fast or helical peptides, and finally elongated peptides. The α-helical peptides (e.g., *scorpions, meganins*, and *LL37*) and β-platelets (e.g., *human alpha and beta-defensin*, *pleistacins*, and *protogrins*) are known as the most frequent AMPs in the world (10). In addition, some peptides do not belong to any of these groups and form their active structure when they interact with the target cell membrane or form a different structure in aqueous or fatty media (9).

It has been reported that peptides can also be effective against viral infections (11, 12). Many studies have reported that some peptides could exhibit strong antiviral activities in both *in vitro* and *in vivo* conditions while in contact with the virus. These peptides can kill viruses in a variety of ways, including inactivation of the virus, prevention of the virus from entering cells, interaction with specific cell receptors, or by blockage of viral proteins that target cells (12, 13). Hence, it seems that the function of the FMD virus can be disrupted by the inhibition of VP1 protein via proper antiviral peptides. In this regard, the present study aimed to select appropriate anti-FMD virus peptides among potential antiviral peptides to block the VP1 protein of the FMD virus.

2. Materials and Methods

2.1. Data collection and antimicrobial possibility investigation

To collect amino acid sequences of potential antiviral peptides, the CAMPR3 database (http://www.camp.bicnirrh.res.in/) was employed. It

must be mentioned that the collection was performed based on length (1-100 mer), experimental validity, and antiviral quality. Moreover, the VP1 gene sequence of serotype O virus isolated in Iran in 2010 (FMDV O/2010/IRN) was obtained from the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/) with HQ663879 accession number. The amino acid sequence of VP1 protein was artificially obtained by CLC Main Workbench software (version 5.5).

To investigate the AMP probability of the potential antiviral peptides, the Antimicrobial Peptide Scanner vr.2 server (https://www.dveltri.com/ascan/v2/ascan.html) was applied with a threshold > 0.5. In this case, the amino acid sequence of each potential antiviral peptide was submitted in FASTA format. Moreover, to increase the sensitivity of the selection, the potential antiviral peptides with a score higher than 0.7 were selected. Finally, the remaining potential antiviral peptides were employed to the next analysis.

2.2 Evaluation of physicochemical features

In the current study, the most important physicochemical features of the remaining potential antiviral peptides, such as pI, GRAVY, instability index, and net charge, were investigated by the ProtParam server *(https://web.expasy.org/protparam/)*. To predict these parameters, the raw amino acid sequence of each peptide was pasted on the server. The peptides whose instability index was more than 40 or loaded with negative or zero net charges were eliminated from the subsequent analysis. Afterward, the hydrophobicity/hydrophilicity parameter was assessed by PEPTIDE 2.0 server *(https://www.peptide2.com/N_peptide_hydrophobicity_hydrophilicity.php)* to filter the remaining potential antiviral peptides from the previous step. Finally, the Protein-Sol server *(https://protein-sol.manchester.ac.uk/)* was employed to evaluate the solubility parameter using the FASTA format of the amino acid sequence of each remaining antiviral peptide.

2.3. Prediction of the tertiary structure

The remained antiviral peptides, which had more than 30% hydrophobic amino acid residues in their structure along with VP1 protein, were modeled using the I-TASSER server (https://zhanggroup.org/I-TASSER/). The best model of VP1 protein by this server, which had the highest confidence score (C-Score), was refined and then validated using GalaxyRefine (http://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE2) and Volume, Area, Dihedral Angle Reporter (http://vadar.wishartlab.com/) servers, respectively.

2.4. Molecular docking

The interaction between the VP1 protein as receptor and the selected antiviral peptides as ligands was separately performed using the ClusPro 2.0 server (https://cluspro.bu.edu/login.php). The ClusPro is a fully automated server used to predict protein-protein interaction. In the first phase, the server used Piper, an FFT-based hard docking program, to create 1,000 low-connection complexes. In the next step, the server categorized the predicted connections and removed those that were more unstable. Finally, the selected complexes were optimized by Monte Carlo simulation (14). Finally, the hydrogen bonds resulting from this interaction were investigated and visualized by LigPlot + and PyMol software.

3. Results

3.1. Data collection and antimicrobial possibility investigation

As mentioned earlier, in the current study, primary antiviral peptide collection was conducted based on length (1-100 mer), experimental validity, and antiviral quality. According to these features, 28 potential antiviral peptides with a length of 25-43 amino acids were selected to be evaluated regarding their ability to inhibit the VP1 protein of the FMD virus (Table 1). Results of the antimicrobial possibility investigation revealed that all potential antiviral peptides had high

probability values (Table 1).

3.2. Assessment of the physicochemical parameters

In the current project, based on the physicochemical parameters predicted by ProtParam online server, *Circulin-D, Circulin-E, Caerin-1.1, Vhl-2, Neutrophil defensin 3, Defensin-6, Mytilin-B, Melittin, Vhl-1, Palicourein, Corticostatin-6, Corticostatin-3 precursor, Corticostatin-4 precursor*, and *Human Defensin-5* peptides were removed from further analysis due to their negative or zero net charge or high instability index (>40) (Table 2). Among the remaining potential antiviral peptides, the highest length, and consequently, the highest molecular weight belonged to the *Ginkbilobin* peptide, while the lowest weight belonged to *Maximin 1* and *Cycloviolin-B* peptides. Moreover, the highest observed pI was related to the *Ginkbilobin* peptide. The highest and the lowest GRAVY parameters were -0.608 and 1.188 which belonged to *Reptilian Defensin* and *Caerin-1.1* peptides, respectively (Table 2).

Table 1. List of potential antiviral peptides

No	Name	UniProt ID	Source	Amino Acid Sequence	AMP Probability
1	*Caerin-1.1*	P62568	Litoria splendida	GLLSVLGSVAKHVLPHVVPVIAEHL	AMP (0.99)
2	*Melittin*	1BH1, 2MLT	Apis mellifera	GIGAVLKVLTTGLPALISWIKRKRQQ	AMP (0.99)
3	*Maximin 1*	P83080	Bombina maxima	GIGTKILGGVKTALKGALKELASTYAN	AMP (0.99)
4	*Cycloviolin-B*	P84638	Leonia cymosa	GTACGESCYVLPCFTVGCTCTSSQCFKN	AMP (1)
5	*Neutrophil antibiotic peptide NP-3 precursor*	Q62713	Rattus norvegicus	CSCRTSSCRFGERLSGACRLNGRIYRLCC	AMP (1)
6	*Circulin-F*	P84644	Chassalia parviflora	AIPCGESCVWIPCISAAIGCSCKNKVCYR	AMP (1)
7	*Neutrophil defensin 3, Defensin-6*	P59666	Homo sapiens	DCYCRIPACIAGERRYGTCIYQGRLWAFCC	AMP (1)
8	*Neutrophil cationic peptide 1 type B preproprotein*	Q64365	Cavia porcellus	RCICTTRTCRFPYRRLGTCIFQNRVYTFCC	AMP (1)
9	*Vhl-2*	P85231	Viola hederacea	GLPVCGETCFTGTCYTNGCTCDPWPVCTRN	AMP (1)
10	*Dermaseptin-3*	P80279	Phyllomedusa sauvagii	ALWKNMLKGIGKLAGKAALGAVKKLVGAES	AMP (0.99)
11	*Cycloviolin-C*	P84639	Leonia cymosa	GIPCGESCVFIPCLTTVAGCSCKNKVCYRN	AMP (1)
12	*Cycloviolin-D*	P84640	Leonia cymosa	GFPCGESCVFIPCISAAIGCSCKNKVCYRN	AMP (1)
13	*Circulin-C*	P84641	Chassalia parviflora	CGESCVFIPCITSVAGCSCKSKVCYRNGIP	AMP(1)
14	*Circulin-D*	P84642	Chassalia parviflora	KIPCGESCVWIPCVTSIFNCKCENKVCYHD	AMP (0.99)
15	*Circulin-E*	P84643	Chassalia parviflora	KIPCGESCVWIPCLTSVFNCKCENKVCYHD	AMP (0.99)
16	*Neutrophil antibiotic peptide NP-4*	Q62714	Rattus norvegicus	ACYCRIGACVSGERLTGACGLNGRIYRLCCR	AMP (1)

	precursor				
17	*Vhl-1*	P84522	Viola hederacea	SISCGESCAMISFCFTEVIGCSCKNKVCYLN	AMP (0.99)
18	*Kalata-B8*	P85175	Oldenlandia affinis	GSVLNCGETCLLGTCYTTGCTCNKYRVCTKD	AMP (1)
19	*Cycloviolin-A*	P84637	Leonia cymosa	GVIPCGESCVFIPCISAAIGCSCKNKVCYRN	AMP (1)
20	*Human Defensin-5*	Q01523	Homo sapiens	ATCYCRTGRCATRESLSGVCEISGRLYRLCCR	AMP (0.99)
21	*Corticostatin-3 precursor*	P01376	Oryctolagus cuniculus	VVCACRRALCLPRERRAGFCRIRGRIHPLCCRR	AMP (1)
22	*Corticostatin-4 precursor*	P01377	Oryctolagus cuniculus	VVCACRRALCLPLERRAGFCRIRGRIHPLCCRR	AMP (1)
23	*Mytilin-B*	P81613	Mytilus edulis	SCASRCKGHCRARRCGYYVSVLYRGRCYCKCLRC	AMP (1)
24	*Corticostatin-6*	P80223	Oryctolagus cuniculus	GICACRRRFCLNFEQFSGYCRVNGARYVRCCSRR	AMP (1)
25	*Reptilian Defensin*	P0CAP0	Caretta caretta	EKKCPGRCTLKCGKHERPTLPYNCGKYICCVPVKVK	AMP (1)
26	*Palicourein*	P84645	Palicourea condensata	GDPTFCGETCRVIPVCTYSAALGCTCDDRSDGLCKRN	AMP (0.99)
27	*Ginkbilobin*	P83171	Ginkgo biloba	ANTAFVSSAHNTQKIPAGAPFNRNLRAMLADLRQNAAFAG	AMP (0.98)
28	*Antihypertensive protein BDS-1*	P11494	Anemonia sulcata	AAPCFCSGKPGRGDLWILRGTCPGGYGYTSNCYKWPNICCYPH	AMP (1)

Table 2. Physicochemical features of potential antiviral peptides

No	Name	Length	MW	pI	GRAVY	Instability index	Net Charge
1	*Dermaseptin-3*	30	3023.72	10.30	0.350	-5.28	+5
2	*Maximin 1*	27	2675.16	9.83	0.252	7.03	+3
3	*Cycloviolin-B*	27	2675.16	9.83	0.252	7.03	+3
4	*Circulin-D*	30	3420.03	6.71	0.113	15.02	0
5	*Circulin-C*	30	3125.72	8.33	0.560	16.17	+2
6	*Circulin-F*	29	3075.70	8.34	0.652	25.80	+2
7	*Circulin-E*	30	3420.03	6.71	0.090	26.79	0
8	*Kalata-B8*	31	3307.81	7.67	-0.023	27.53	+1
9	*Cycloviolin-A*	31	3235.88	8.33	0.681	28.76	+2
10	*Neutrophil antibiotic peptide NP-4 precursor*	31	3338.97	8.96	0.303	28.82	+4
11	*Antihypertensive protein BDS-1*	43	4714.42	8.64	-0.309	29.18	+3
12	*Caerin-1.1*	25	2585.13	7.02	1.188	29.70	0
13	*Reptilian Defensin*	36	4080.98	9.39	-0.608	31.60	+7
14	*Cycloviolin-C*	30	3166.77	8.33	0.450	32.98	+2
15	*Cycloviolin-D*	30	3170.76	8.33	0.507	36.77	+2
16	*Ginkbilobin*	40	4213.75	11.71	-0.180	37.12	+3
17	*Neutrophil antibiotic peptide NP-3 precursor*	29	3271.83	9.25	-0.169	37.39	+5
18	*Vhl-2*	30	3199.63	4.37	-0.043	39.21	-1
19	*Neutrophil cationic peptide 1 type B preproprotein*	30	3682.39	9.49	-0.057	39.35	+6
20	*Neutrophil defensin 3, Defensin-6*	30	3492.10	8.33	0.123	41.06	+2
21	*Mytilin-B*	34	3981.76	9.58	-0.344	44.30	+9
22	*Melittin*	26	2847.49	12.02	0.273	44.73	+5
23	*Vhl-1*	31	3340.94	5.85	0.690	56.12	0
24	*Palicourein*	37	3928.43	4.78	-0.189	60.26	+1
25	*Corticostatin-6*	34	4051.74	9.84	-0.344	69.40	+7
26	*Corticostatin-3 precursor*	33	3897.79	11.40	-0.112	84.77	+9
27	*Corticostatin-4 precursor*	33	3854.76	11.12	0.139	87.05	+8
28	*Human Defensin-5*	32	3588.19	8.96	-0.113	1379	+4

3.3. Hydrophobicity and solubility

As it is summarized in table 3, the Protein-Sol server determined that all the remaining peptides from the previous step were soluble (Table 3). Hydrophobicity of the selected peptides from the previous step was successfully determined using the PEPTIDE 2.0 server. As reported in table 3, the selected peptides were sorted according to their number of hydrophobic amino acids (%). The results demonstrated that the *Dermaseptin-3* peptide with 53.33% of hydrophobic amino acid residues contained the highest number of hydrophobic amino acids, while the *Kalata-B8* peptide with 16.13% had the lowest number of hydrophobic amino acid residues (Table 3).

3.4. Determination and Validation of Tertiary Structures

Tertiary structures of *Dermaseptin-3, Ginkbilobin, Circulin-F, Maximin 1, Cycloviolin-A, Cycloviolin-D, Circulin-C,* and *Cycloviolin-C* and *Antihypertensive protein BDS-1* peptides (as final antiviral peptides), as well as VP1 protein, were modeled with -0.43, -1.41, 0.31, -0.60, 0.61, 0.64, 0.87, 0.68, -0.53 and 1.30 C-score, respectively. In the next step, to achieve the most accurate folded state of the presented VP1 model, the GalaxyRefine server was employed to refine the best model and then was verified using Ramachandran analysis. In the refined model of VP1 protein, 93%, 5%, 0%, and 0% of the residues were located in the core, allowed, generous, and outside regions, respectively, while these indexes in the primary model predicted by the I-TASSER server were 74%, 22%, 3%, and 0%, respectively (Figure 1).

3.5. Molecular docking

Investigations were conducted to examine the possible interaction between the VP1 protein and final antiviral peptides with more than 30% hydrophobic amino acid residues content (including *Dermaseptin-3, Ginkbilobin, Circulin-F, Maximin 1, Cycloviolin-A, Cycloviolin-D, Circulin-C, Cycloviolin-C,* and *Antihypertensive protein BDS-1* peptides) using the ClusPro 2.0 server. Results of molecular docking showed that the lowest energy levels of docking between VP1 protein and *Dermaseptin-3, Ginkbilobin, Circulin-F, Maximin 1, Cycloviolin-A, Cycloviolin-D, Circulin-C, Cycloviolin-C,* and *Antihypertensive protein BDS-1* peptides were -743.4, -1295.4, -856.3, -715.2, -866.2, -865.0, -814.4, -864.0, and -951.9 kcal/mol, respectively.

Table 3. Assessment of the hydrophobicity and solubility of the potential antiviral peptides

Name	Scaled Solubility	Hydrophobic %	Acidic %	Basic %	Neutral%
Dermaseptin-3	0.774 (Soluble)	53.33	3.33	20	23.33
Ginkbilobin	0.638 (Soluble)	52.5	2.5	12.5	32.5
Circulin-F	0.798 (Soluble)	41.38	3.45	10.34	44.83
Maximin 1	0.710 (Soluble)	40.74	3.7	14.81	40.74
Cycloviolin-A	0.827 (Soluble)	38.71	3.23	9.68	48.39
Cycloviolin-D	7.03 (Soluble)	36.67	3.33	10	50
Circulin-C	0.818 (Soluble)	33.33	3.33	10	53.33
Cycloviolin-C	0.818 (Soluble)	33.33	3.33	10	53.33
Antihypertensive protein BDS-1	0.707 (Soluble)	32.56	2.33	11.63	53.46
Neutrophil antibiotic peptide NP-4 precursor	0.631 (Soluble)	29.03	3.23	16.13	51.61
Reptilian Defensin	0.863 (Soluble)	27.78	5.56	27.78	38.89
Neutrophil cationic peptide 1 type B preproprotein	0.627 (Soluble)	26.67	0	20	53.33
Cycloviolin-B	0.606 (Soluble)	25	3.75	3.57	67.86
Neutrophil antibiotic peptide NP-3 precursor	0.663 (Soluble)	20.69	3.45	20.69	55.17
Kalata-B8	0.715 (Soluble)	16.13	4.45	9.68	67.74

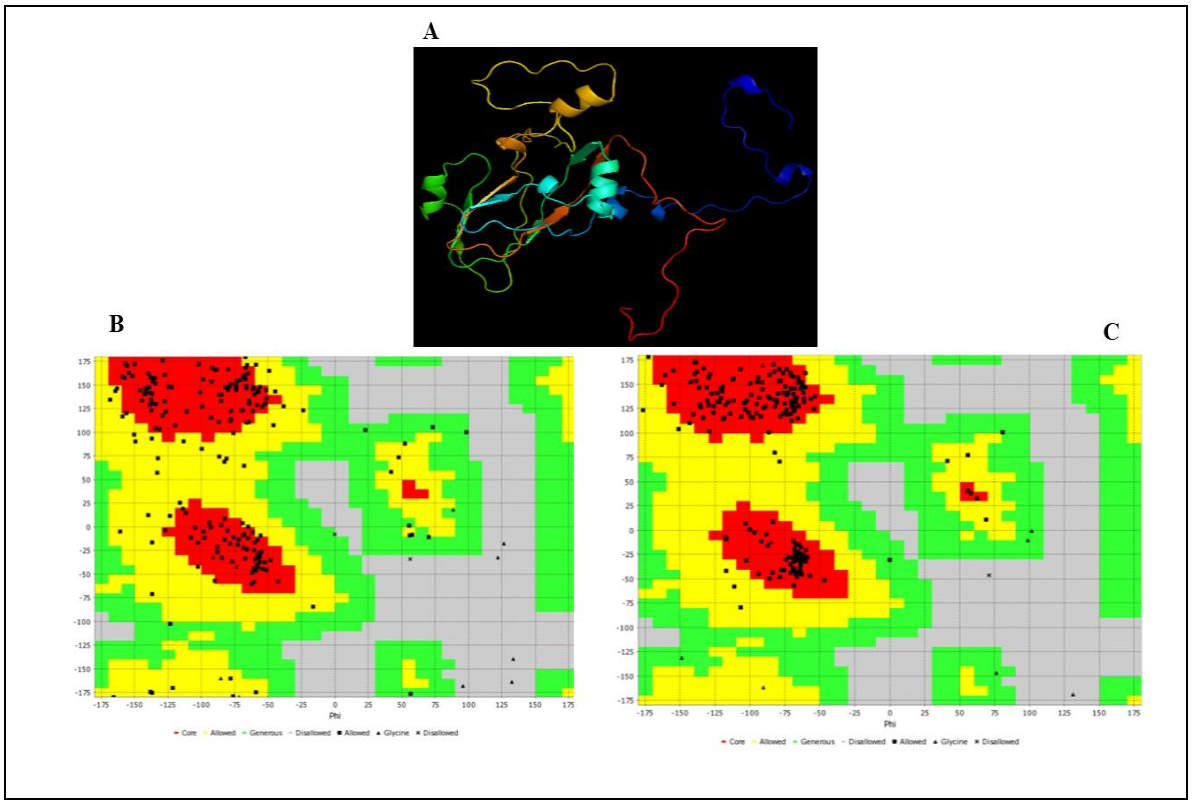

Figure 1. A: tertiary structure of the VP1 protein modeled by I-TESSAR server. **B**: Ramachandran plot of the primary model of VP1 protein. In this model, 74%, 22%, 3% and 0% of the residues were located in core, allowed, generous and outside regions, respectively. **C**: Ramachandran plot of the refined model of VP1 protein. In this model, 93%, 5%, 0% and 0% of the residues were located in core, allowed, generous and outside regions, respectively

Further analysis was performed to determine the number and length of hydrogen bonds using LigPlot+ software. Based on the results, among the mentioned peptides, *Dermaseptin-3* (Figure 2) and *Ginkbilobin* peptides (Figure 3) formed the highest number of hydrogen bonds (10 bonds) with nine and seven amino acid residues from VP1 protein, respectively (Table 4). In the next rank, *Cycloviolin-A* and *Circulin-C* peptides were also able to establish nine hydrogen bonds with eight and seven amino acids of VP1 protein, respectively. In addition, *Circulin-F* and *Maximin I* peptides with two and three hydrogen bonds showed the lowest number of bindings with VP1 protein, respectively. (Table 4).

4. Discussion

Bioinformatics is an interdisciplinary science that includes methods and software used for understanding biological information. The increasing volume of genomic data and the need to store, retrieve, and properly analyze this data has led to the emergence of bioinformatics (15). Today, this knowledge, as a powerful science, leads to the production of highly accurate data, at a much lower cost, compared to an *in vitro* study. In fact, bioinformatics analyzes biological data that is used in various fields of biology, such as drug discovery and vaccine design (16). In this regard, the study of the structure and activity of macromolecules in different conditions and also interactions between them is necessary to better understand cellular function at the molecular level in the biological sciences. (17). Therefore, as a method based on the investigation of the structure of the protein-protein complex in drug design, molecular docking is a key tool in the structural molecular biology and computational design of drugs.

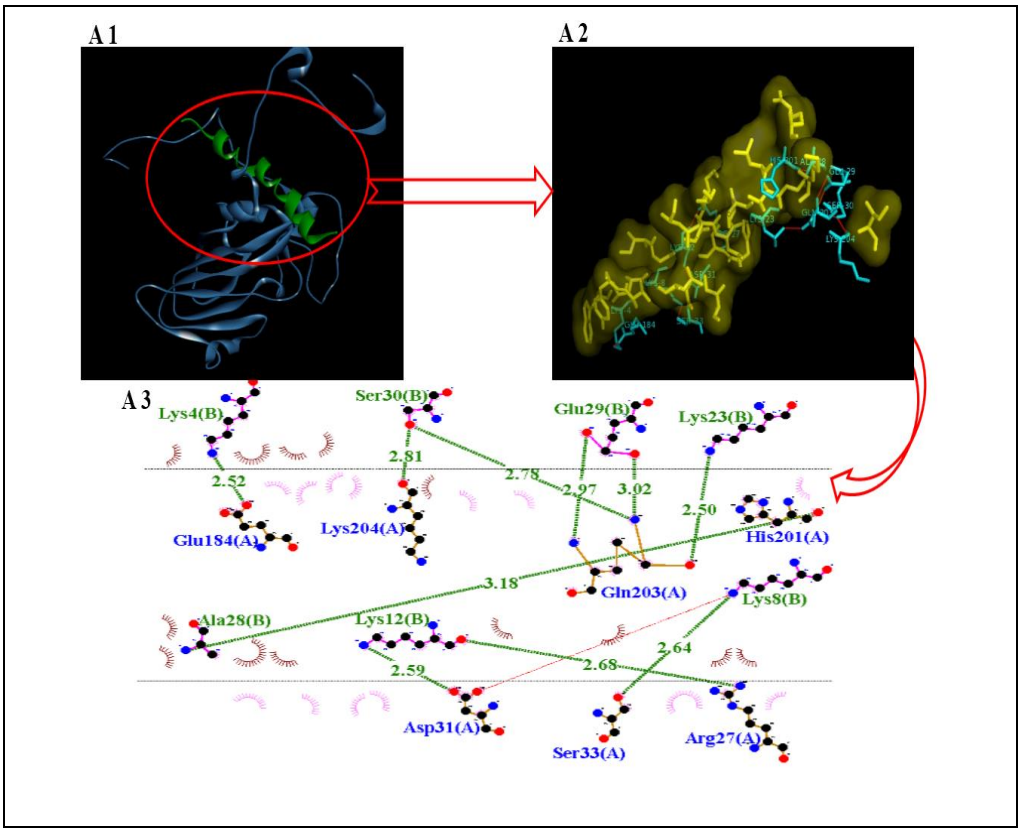

Figure 2: (**A1)** Interaction between *Dermaseptin-3* peptide (the green color) and VP1 protein (the blue color) which was created by ClusPro server and visualized by PyMol software. **(A2)** The exact docked region which was visualized by PyMol software (the blue color refers to amino acid residues which take part in hydrogen bond formation and the red color represents the hydrogen bonds). (**A3)** The docked regions which were analyzed by LigPlot + software (the green amino acids (B) belong to *Dermaseptin-3* peptide, the blue amino acids (A) belong to VP1 protein and the green dotted lines represent hydrogen bonds)

The purpose of ligand-protein binding is to predict the state or states of the major ligand binding to a protein with a known three-dimensional structure (18). Today, the application of such bioinformatic techniques in virtual screening or de-novo design, before the preparation and synthesis of biomolecules in *in vitro* studies, has received a lot of attention from researchers as it allows them to enter the experimental phase of research with a valuable perspective of the function of peptides and proteins. (19-21). Usage of antibiotics is usually the first option in disease treatment with a microbial origin; however, today, the issue of antibiotic resistance is very worrying. Microorganisms can show resistance to antibiotics using several methods, including the following: (1) limiting the use of antibiotics by microorganisms through the reduction of their permeability and alteration of their hydrophobic properties (22); (2) changing the goals of drugs when antibiotics target multiple sites in a cell (23); (3) inactivating antibiotics through the enzymatic mechanism of microorganisms (24); and (4) overexpressing transporter genes and efflux pumps that divert the path of the antibiotic to its target position in the microorganism (25). Therefore, antibiotic resistance is one of the most important challenges of the treatment. The AMPs are natural oligo-peptides that belong to inherently immune components of organisms and have significant action against pathogens. Due to their significant antibacterial and antiviral activities, it has recently been reported that AMPs have received more

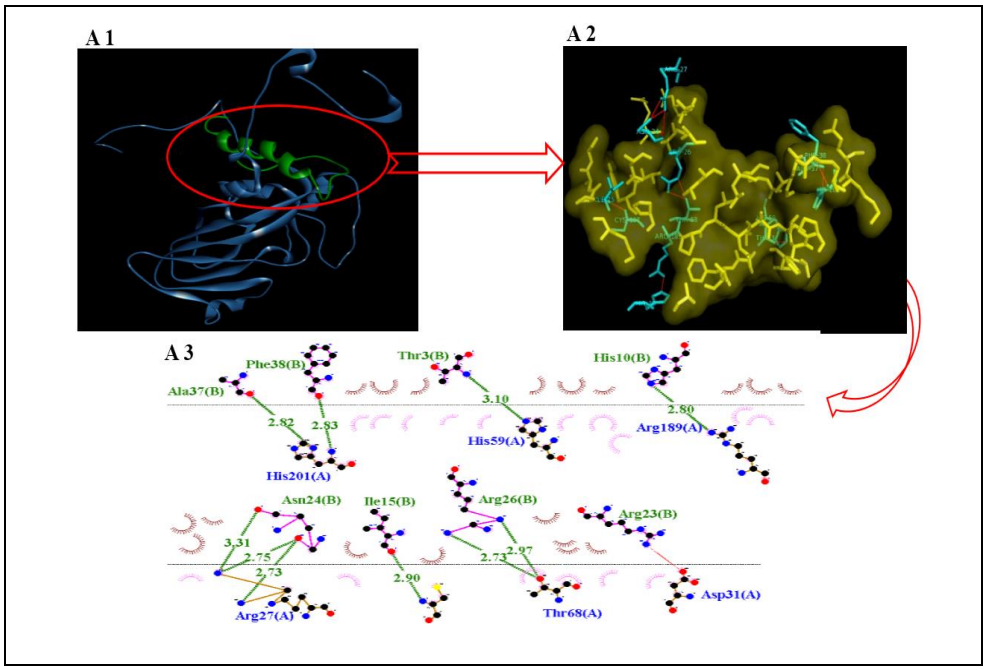

Figure 3: (A1) A scheme of docking the *Ginkbilobin* peptide (the green color) and VP1 protein (the blue color) which was visualized by PyMol software. (**A2)** The region for establishing hydrogen bonds between two domains which was visualized by PyMol software (the blue color is related to the amino acids that participated in the establishment of the hydrogen bond and the red color of the hydrogen bonds). (**A3)** A scheme of hydrogen bonds (the green dotted line) between the amino acids of the *Ginkbilobin* peptide (the green amino acids (B)) and VP1 protein (the blue amino acids (A)) which was analyzed by LigPlot + software

Table 4. List of amino acids involved in hydrogen bonds between final antiviral peptides and VP1 protein

Name	Amino acid of antiviral peptide	Amino acid of VP1 protein	Length of hydrogen bond (Aº)
Circulin-F	Ala 17	His 195	2.98
	Trp 10	*Arg 157	2.82
	Gly 6	Asn 131	2.80
	Ser 8	Arg 189	2.84
	Ser 8	Tyr 71	2.90
	Glu 7	Tyr 71	3.28
Cycloviolin-A	Arg 30	Tyr 130	2.74
	Ile 15	His 123	8.87
	Tyr 29	*Asn 143	3.22
	Val 2	*Arg 140	2.66 2.81
	Lys 4	Glu 184	2.52
	Ser 30	Lys 204	2.81
	Ser 30	Gln 203	2.78
	Glu 29	Gln 203	2.97 3.02
Dermaseptin-3	Lys 23	Gln 203	2.50
	Ala 28	His 201	3.18
	Lys 12	Asp 31	2.59
	Lys 12	Arg 27	2.68
	Lys 8	Ser 33	2.64
	Ala 37	His 201	2.87
	Phe 38	His 201	2.83
	Thr 3	His 59	3.10
Ginkbilobin	His 10	Arg 189	2.80
	Asn 24	Arg 24	2.73 2.75

	Ile 15	Cys 187	3.31 2.90
	Arg 26	Thr 68	2.97 2.73
	Tyr 25	Cys 134	3.12
Maximin I	Tyr 25	Asp 133	2.83
	Tyr 25	Lys 135	2.94
	Ala 17	Tyr 136	2.72
	Il 18	Tyr 130	2.97
Cycloviolin-D	Glu 6	Asn 131	3.00 3.08
	Gly 1	*Arg 140	2.95 2.66
	Cys 4	Lys 154	2.73
	Ser 21	Gln 25	3
	Lys 20	Gln 25	3.19
	Ile 29	Arg 26	2.74
Circulin-C	Tyr 25	Arg 26	2.80 2.76
	Pro 30	Arg 26	2.88 2.69
	Gly 28	Arg 26	2.68
	Ser 4	Thr 30	2.87
	Phe 10	*Arg 140	2.69 2.90
	Glu 6	Asp 131	3.21
	Ser 7	Tyr 130	2.80
Cycloviolin-C	Cys 4	His 123	2.95
	Arg 29	Tyr 186	2.91
	Gly 1	Arg 124	2.70 2.90
	Pro 3	Arg 124	2.71
	Ala 2	His 29	3.12
	Arg 19	Tyr 68	3.07
Antihypertensive protein BDS-1	Gly 27	Arg 200	3.28 2.69
	Phe 5	Gln 25	3.10
	Asn 37	Arg 27	2.62
	Ser 7	Ala 13	3.01

*The amino acid residues which are present in G-H loop motif

attention as a safer alternative for the treatment of diseases caused by bacteria or viruses (26). Until now, the importance of using AMPs as a safe treatment with the least side effects has been proven in many studies, such as using *Mycobacterium leprae* in human skin diseases in Leprosy disease, pig *cathelicidin PR-39* in skin lesions, and *Staphylococcus aureus* in atopic dermatitis (27). In another study, researchers have proved that a synthesized form of the *Epinephelus coioides* antimicrobial peptide, epinecidin-1 (Epi-1), could combat FMDV. The Epi-1 is known to have broad-spectrum antimicrobial activity and low toxicity toward normal eukaryotic cells, making it a good candidate for use as a therapeutic agent (28). The FMD is one of the most contagious viral diseases among livestock, which ranks first in the list of infectious diseases of animals. Despite low mortality rates, it has been reported that FMD drastically reduces livestock production and imposes significant trade restrictions on livestock and livestock products (29, 30). The FMD (FMDV, family *Picornaviridae*, genus *Aphthovirus*), is structurally distinct from other members of its family due to the presence of a G-H-protruding loop from the VP1 protein. The G-H loop is a motif consisting of 20 amino acid residues in the range 140-160 (31). Based on the results of this research, *Cycloviolin-A*, *Dermaseptin-3*, *Ginkbilobin*, and *Circulin-C* peptides with 9, 10, 10, and 9 hydrogen bonds, respectively, showed the strongest bonds with the VP1 protein. Among these peptides, *Cycloviolin-A* peptide, through its numerous hydrogen bonds, has been able to inhibit the Asn 143 and Arg 140 amino acid residues of the protein and VP1 by hydrogen bonding with a length of 3.22 Aº and 2.81 Aº, respectively. These amino acids are located in the G-H loop (in the range of 140-160 amino acid residuals), which seems to have a more favorable effect on the inhibition of VP1 protein binding to the cell wall. Therefore, based on the reported results in the present study, it can be concluded that the *Cycloviolin-A*, *Dermaseptin-3*, *Ginkbilobin*, and *Circulin-C* peptides could be suitable and promising candidates for the drug design to combat the dangerous FMD disease. The current study was designed to identify specific anti-FMD virus peptides. In this case, 28 potential antiviral peptides were collected from the database and the most important features of these peptides were investigated by reliable servers and software. The results of this study revealed that *Dermaseptin-3* and *Ginkbilobin* peptides were able to strongly inhibit the VP1 protein

using 10 hydrogen bonds. Therefore, these peptides were introduced as promising candidates to prevent the FMD virus.

Authors' Contribution

The project was designed by Ali Forouharmehr, whereas Narges Nazifi and Amin Jaydari conducted the different analyses.

Ethics

This article does not consist of any studies with animals performed by any of the authors.

Conflict of Interest

The authors declare there is no conflict of interest.

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