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Research Article

IDENTIFICATION OF STABLE ANTI-Viral PEPTIDES FOR THERAPEUTIC APPLICATIONS - A COMPUTATIONAL APPROACH

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ABSTRACT

With the passage of time, clinically significant antibiotic resistance has evolved globally against almost every antibiotic deployed. Yet the development of new classes of antibiotics has lagged far behind our growing need for such new and effective drugs. Antimicrobial peptides (AMPs) have emerged as novel therapeutics hailed for their virucidal**,** bactericidal and immunomodulatory properties. However, the process of optimizing antimicrobial peptide stability using large peptide libraries is both tedious and expensive. The intent of this study is to analyze computationally the stability of Anti-viral peptides (AVPs) and to discover a potential candidate from a pool of AVPs for therapeutic use. Consequently we highlighted that the AVP human α-defensin-5 (HD5) appears advantageous over the other AVPs used in the study with respect to stability and may provide a convenient platform for the development of a suitable anti-viral therapeutic peptide.

Keywords: Anti-viral peptides; Human α-defensin; Stabilization centers; Total energy; Cation-π interactions; Non-covalent and non-canonical interactions; Instability index.

INTRODUCTION

A key challenge to the antibiotics industry is that constant innovation is necessary not only because of development of resistant strains, but also because of side effects. The drastic increase in microbial pathogens, which are resistance to conventional antibiotics, is a global public health problem [1-7]. Therefore, the search continues for new antibiotics that are active *in vivo*, are fast acting and broad-spectrum, do not induce resistance and have limited side effects. Antimicrobial peptides (AMPs) represent such a new class of antibiotics. Synthetic congeners of natural antimicrobial peptides are good candidates. In addition they display many more advantages like low MICs (i.e. minimum inhibition concentrations) and broad-spectrum activity in both low and high ionic strength conditions [8]. Several AMPs have already entered pre-clinical and clinical trials to promote wound healing [9-10], for the treatment of cystic fibrosis, catheter site infections, acne and patients undergoing stem cell transplantation [11-13]. The AMPs also have synergistic activity with conventional antibiotics [14]. Recently Fehri et al. [15] have reported that there is a synergistic action between the fluoroquinolone enrofloxacin (EFX) and two AMPs (alamethicin and surfactin). The combination of AMPs and conventional antibiotics required less concentration of the antibiotic than its normal MIC and also the action was much faster than that of conventional antibiotics or AMPs working alone.

Although AMPs are a focus of biotechnological research and development, recent studies on their anti-viral activities are exciting. AVPs are ubiquitous and simple. They have been isolated from all animals and plants in which they have been sought and so far several hundred AVP sequences have been characterized in higher organisms [16]. Mode of action of AVPs varies from one peptide to other. Some of the common mechanisms involved in AVP actions are b locking of viral entry by heparan sulfate interaction, blocking of viral entry by interaction with specific cellular receptors, blocking of viral entry by interaction with viral glycoproteins, membrane or viral envelope interaction, intracellular targets and host cell stimulation[17]. Their short length along with their fast and efficient action against viruses has made them potential candidates as peptide drugs[18-25]. Apart from the above stated advantages of using AMPs as therapeutics, one major advantage is that they are multifunctional[26]. Besides their antimicrobial activity, they have multiple roles as mediators of inflammation influencing diverse processes such as cell proliferation and migration, immune modulation, wound healing, angiogenesis and the release of cytokines and histamine.

Most natural AMPs are cationic and ampipathic, these features promote interaction with the negatively charged cell membranes. Although AMPs are broad-spectrum antimicrobials displaying rapid bactericidal activity, from the perspective of therapeutic applications, the activities of AMPs can be hampered *in vivo* by different biological factors, such as enzymatic cleavage (notably for natural AMPs composed of L-α-amino acid residues' susceptible to proteolysis) or absorption by serum lipoproteins. Because of low stability and selectivity, a high dosage of the peptide is essential in order to elicit the proper therapeutic effects which is often not economical and is beyond the clinically acceptable level[27-28]. This has created the need for the discovery of synthetic peptide. Furthermore, synthetic peptides have altered sequences to decrease recognition by proteases and enhance biological activity [29]. Hence, a better knowledge of the structure-activity and structure-stability relationships of AMPs makes it feasible to design synthetic AMPs with a better therapeutic index than natural AMPs.

Development of strains resistant to antimicrobial peptides from previously sensitive strains has been viewed as difficult with comparision to development of resistance to currently marketed antibiotics[30-31]. So AMPs are better in terms of development of resistance. Moreover, as these peptides tend to be short (< 40 residues), thus they are easily accessible to solid phase synthesis and in terms of economy of production once cloned into competent cells, thus ready for production by fermentation they become more economical, less labour and time consuming than most marketed chemically manufactured antibiotics.

Researchers at Wisconsin Alumni Research Foundation (WARF) have introduced a synthetic peptide that shows antiviral activity against influenza viruses by inhibiting the attachment of influenza virus to host cells, preventing viral infection [32]. Moreover, a recent study showed that synthetic peptides, most notably P1 and P9, inhibited West Nile virus (WNV) infection of cells *in vitro*[33]. P9 also protected mice from lethal infection with WNV, and P1 demonstrated *in vitro* inhibition against dengue virus. Although synthetic peptides may have several advantages, with the association of computational biology one may expect still better achievements in the synthetic peptide development. Hence, in the present study, we have identified the molecular level stability for all the AVPs structure available in the database with the help of standard computational algorithms. According to the Science Citation Index (July 1995), the computational parameters such as SCide, CaPTURE, WHAT IF and Instability Index program has been cited in the scientific literature more than 100 times. We believe that this track should be of immense importance in the identification AVPs with better *in vivo* stability. This could also act as a template for designing peptide drug against viral pathogens.

MATERIALS AND METHODS

Data set

We have selected a set of 31 non redundant anti-viral peptides from updated APD2 database for our investigation[34]. Therapeutic peptides should be designed based on 'templates derived from naturally occurring antimicrobial peptides. The present study also focuses only the naturally occurring antiviral peptides which could be obtained from APD2 database. The results from the experimental analysis also prove the principle that APD2 is a useful tool for peptide design[34]. In particular, APD2 collects only 'mature and active' peptides. Hence, only APD2 database were considered during the data collection. The APD2 database possesses a total of 1746 peptide entries (By March 2011) of which 103 is the AVPs. In these 103 entries only 31 peptides have known three dimensional structures[34]. That is why we have selected these 31 peptides for our investigation. The AVPs chosen for the study represents a wide spectrum of structural families. The set includes five groups of AVPs such as α-Helical, β-structure without di-sulfide bonds, combined α-Helical, β-structure, Rich in specific residues and β-structure with di-sulfide bonds. This shows that the selected set of proteins is representative and that it forms a solid basis for statistical analysis. The three dimensional structure of these AVPs were retrieved from Protein Data Bank (PDB)[35]. The PDB id's are as follows: 1VM5, 1LFC, 1RPB, 1F0F, 2MLT, 1G89, 3GNY, 3HJ2, 1DFN, 1ZMM, 1ZMP, 1ZMQ, 1PG1, 1RKK, 1WO1, 1BH4, 2ERI, 1KJ6, 2K6O, 1ZRV, 1HVZ, 1E4S, 1ZFU, 2FBS, 1KAL, 1K48, 1R1F, 1ZA8, 2GJ0, 2ATG and 2B5B. The approach implemented to identify therapeutic anti-viral template from the pool of AVPs is shown in Figure 1.

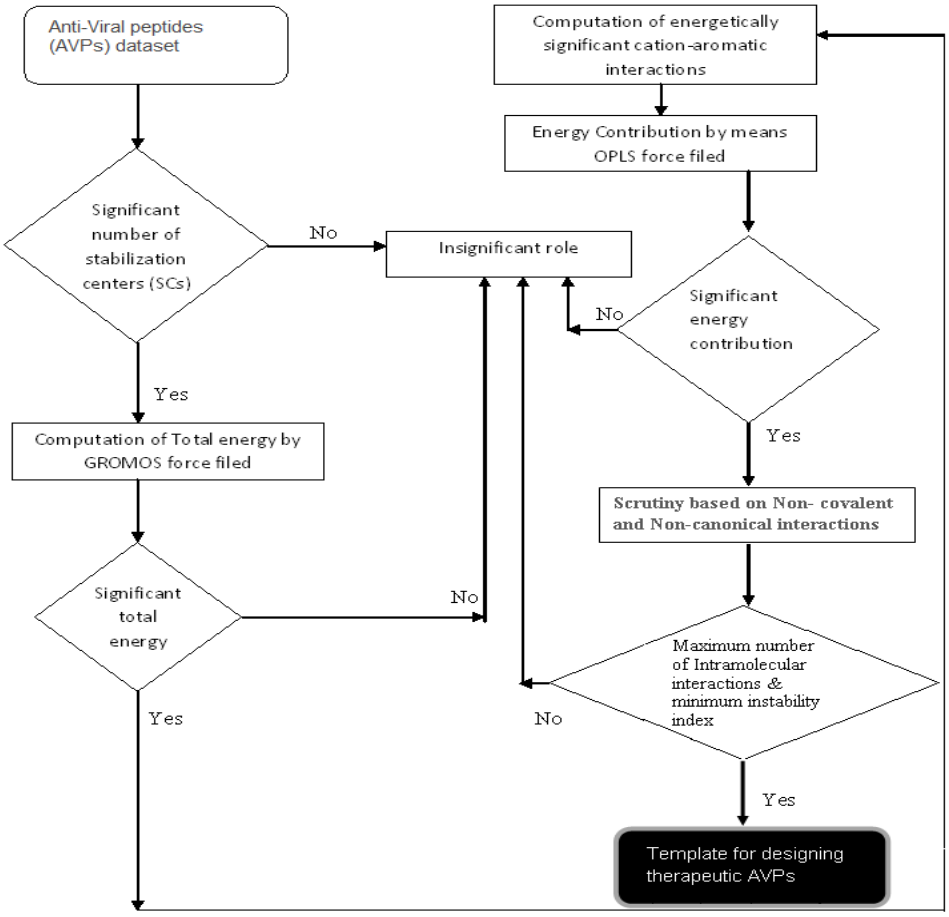

Fig. 1: Schematic representation of the different parameters considered to identify potential therapeutic template.

Consensus approach to identify Stabilization Centers and Total Energy

A consensus approach has been developed for locating the stabilization centers[36]. SCs are clusters of residues involved in long range interactions. Two residues are considered to be in long-range interaction if they are separated by at least 10 residues in the sequence and at least one of their heavy-atom contact distances is less than the sum of their Van der Waals radii of the two atoms, plus 1 Å. Two residues are part of stabilization centers if (i) they are involved in long range interactions and (ii) two supporting residues can be selected from both of their flanking tetra peptides, which together with the central residues form at least seven out of the possible nine contacts. We have obtained the information about the SCs using SCide program[37]. Then, the total energy calculations for all the peptides were carried out . Total energy includes bonds, angles, torsions, improper, non bonded and electrostatic constraints and was calculated from GROMOS force field implemented in Deep View[38] for AVPs to check their stability.

Energetic contribution due to cation–π interactions

Cation–π interaction energies are obtained using the CaPTURE program (cation–π trends using realistic electrostatics)[39]. The energies are computed for all the pairs of cationic-aromatic amino acid residues (Arg/Lys with Phe, Tyr and Trp). The total cation–π interaction energies ($E_{cat-\pi}$) are divided into electrostatic (E_{es}) and van der Waals energies (E_{vdw}). The program implements a variant of the OPLS force field to identify potential cation–π interactions using an energy threshold[39]. For the residues to qualify as cation–π interacting pairs, they must be within 10Å of each other and less than 2.8Å (size of a water molecule) at their closest approach[39]. Also, the electrostatic energy (E_{es}) must be less than-2 kcal/mol or both the E_{es} and E_{vdw} must be below -1 kcal/mol[39].

Non-Covalent Interaction and Non-Canonical Interaction Analysis

The three-dimensional structure of a peptide or protein is determined by its covalent structure, i.e. its amino acid sequence, the forces responsible for the folding and stabilization of the structure are mainly non-covalent in nature. These non-covalent interactions include hydrogen bonds (H-bonds), hydrophobic interaction, disulphide bridges and salt bridges[40-46]. In addition to these non-covalent interactions, non-canonical interactions like C–H...π, C-H...O and N-H…π interaction have been recognized as important interaction in protein stability analysis[47-51]. We used the program WHAT IF[52] to find out the number of contact involved in S-S bridges, salt bridges, hydrophobic interactions, Ball View[53-54] for computing the number of contacts in hydrogen bonding for a given polypeptide structure and HBAT program[55] for computing the number of C–H...π, C-H...O and N-H…π interaction in the given AVPs structure.

Instability index

The protein stability in the test tube could be successfully examined by using the Instability index parameter[56]. The experimental work of Guruprasad et al. with 12 unstable and 32 stable proteins by revealed that there are certain dipeptides, the occurrence of which is significantly different in the unstable proteins compared with those in the stable ones. Based on the impact of these dipeptides on the unstable proteins over the stable ones, a weight value of instability is assigned to each of the dipeptides. For a given protein the summation of these weight values normalized to the length of its sequence helps to distinguish between unstable and stable proteins. Using these weight values it is possible to compute an instability index (II) which is defined as

$$II = \left(\frac{10}{L}\right) x \sum_{i=1}^{i=L-1} \{DIWV(x[i]x[i+1])\}$$

Where, L is the length of sequence

DIWV(x[i]x[i+1]) is the instability weight value for the dipeptide starting in position i.

A protein whose instability index is smaller than 40 is predicted as stable, a value above 40 predicts that the protein may be unstable.

RESULTS

Computation of stabilization centers and total energy for AVPs

Identifying the SCs and total energy allows biologist to quickly annotate the stability of AVPs structures. The stabilization centers for all the AVPs structure was identified using the program Scide[37]. This observation indicates that 74 percentage of AVPs in the data set exhibit stabilization centers. In particular, 55 percentages of AVPs having more than 5 stabilization centers and 19 percentages of AVPs show less than 5 stabilization centers (Fig. 2). The details of the stabilization centers in each AVP structures were depicted in Table 1.

Table 1 List of stabilization centers in the AVPs data set.

PDB Code	Stabilization centers (SCs)
1BH4	Gly (2), Glu (3), Ser (4), Cys (5), Val (23), Cys (24), Tyr (25).
1DFN	Chain A: Tyr (4), Cys(5), Arg (6), Arg (15), Arg (16), Tyr (17), Gly (18), Thr (19), Ala (28), Phe(29), Cys (30). Chain B: Tyr(4), Cys (5), Arg (6), Ala (9), Gly (18), Thr (19), Ala (28), Phe (29).
1E4S	Cys (44), Leu (45), Thr (53), Lys (54), Gln (56), Lys (65), Cys (66), Cys (67).
1HVZ	Phe (2), Cys (3), Arg (4), Cys (14), Ile (15), Cys(16), Thr (17).
1K48	Gly (9), Leu (10), Pro (11), Val (12), Thr (16), Val (33), Thr (35).
1KAL	Pro (3), Val (4), Cys (5), Thr (6), Glu (15), Thr (16), Cys (17), Thr (28).
1KJ6	Ala (19), Glu (27), Glu (28), Gln (29), Ile (30), Lys (39), Cys (40), Cys (41), Arg (42), Arg (43).
1LFC	Cys (3), Arg (4), Arg (5), Trp (6), Gln (7), Trp (8), Ile (18), Thr (19), Cys (20), Val (21), Arg (22).
1PG1	Arg (4), Leu (5), Cys (15), Val (16), Gly (17).
1R1F	Phe (2), Cys (3), Glu (5), Thr (6), Cys (7), Gly (29), Leu (30), Cys (31), Lys (32), Arg (33).
1RPB	Leu (2), Ile (4), Gly (5), Ser (6), Cys (7), Asn (8), Tyr (15), Ala (16), Val (17), Cys (19), Phe (20).
1ZA8	Ser (4), Cys (5), Val (24).
1ZFU	Phe (2), Asn (5), Cys (15), Gly (27), Gly (28), Gly (34), Phe (35), Cys (39).
1ZMM	Chain A: Ser (3), Cys (4), Arg (5), Leu (14), Arg (15), Val (16), Gly (17), Asn (18), Thr (27), Tyr (28), Cys (29), Cys (30). Chain B: Ser (3), Cys (4), Arg (5), Gly (17), Asn (18), Thr (27),Tyr (28). Chain C: Ser (3), Cys (4), Arg (5), Leu (14), Arg (15), Val (16), Gly (17), Thr (27), Tyr (28), Cys (29), Cys (30). Chain D: Ser (3), Cys (4), Arg (5), Glu (13), Leu (14), Gly (17), Asn (18), Thr (27), Tyr (28), Cys (30), Thr (31).
1ZMP	Chain A: Tyr (4), Cys (5), Arg (6), Thr (7), Arg (9), Ser (15), Leu (16), Gly (18), Val (19), Tyr (27), Arg (28), Leu (29), Cys (30). Chain B: Tyr (4), Cys (5), Arg (6), Arg (9), Ser (15), Leu (16), Gly (18), Val (19), Arg (28), Leu (29), Cys (30). Chain C: Thr (2), Tyr (4), Cys (5), Arg (6), Thr (7), Arg (9), Ser (15), Leu (16), Gly (18), Val (19), Tyr (27), Arg (28), Leu (29), Cys (30). Chain D: Cys (3), Tyr (4), Cys (5), Arg (6), Ser (17), Gly (18), Val (19), Arg (28), Leu (29), Cys (30).
1ZMQ	Chain A: Cys (4), His (5), Cys (6), Arg (7), Arg (8), Tyr (15), Ser (16), Gly (18), Thr (19), Thr (21), Arg (28), Phe (29), Cys (30). Chain B: His (5), Cys (6), Arg (7), Tyr (15), Ser (16), Gly (18), Thr (19), Cys (20), Arg (28), Phe (29), Cys (30). Chain C: Phe (2), Cys (4), His (5), Cys (6), Arg (7), Arg (8), Ser (9), Tyr (15), Ser (16), Gly (18), Thr (19), Thr (21), Val (22), Arg (28), Phe (29), Cys (30). Chain D: His (5), Cys (6), Arg (7), Arg (8), Ser (9), Tyr (15), Ser (16), Gly (18), Thr (19), Cys (20), Arg (28), Phe (29), Cys (30).
2ATG	Ile (3), Cys (4), Arg (5), Ile (16), Cys (17).
2B5B	Pro (5), Gly (6), Arg (7), Cys (8), Leu (10), Lys (11), Cys (12), Pro (18), Cys (24), Gly (25), Ile (28), Cys (29), Cys (30), Val (31), Pro (32), Val (33), Lys (34), Val (35).
2ERI	Ser (4), Cys (5), Val (23).
2GJ0	Ser (2), Ser (9), Leu (27), Ala (29), Lys (30).
2MLT	Chain A: Leu (6), Leu (9) Chain B: Ile (20).
3GNY	Chain A: Tyr (3), Cys (4), Arg (5), Ala (8), Gly (17), Thr (18), Ala (27), Phe (28) Chain B: Tyr (3), Cys (4), Arg (5), Ala (8), Gly (17), Thr (18), Ala (27), Phe (28).
3HJ2	Chain A: Cys (5), Tyr (6), Cys (7), Arg (8), Ala (11), Gly (20), Thr (21), Ala (30), Phe (31)

Chain B: Ala (4), Tyr (6), Cys (7), Arg (8), Ala (11), Gly (20), Thr (21), Ala (30), Phe (31).

GROMOS force field approach has been applied to determine the total energy of AVPs. The results of total energy for all the AVPs investigated in this study shown in Table 2. It indicates that 7 AVPs (3GNY, 3HJ2, 1DFN, 1ZMM, 1ZMP, 1ZMQ and 1KJ6) exhibits lower energy as compared to the other 11 AVPs (2MLT, 1PG1, 1RKK, 1WO1, 2ERI, 2K60, 1ZRV, 1HVZ, 2FBS, 1 ZA8 and 2ATG). Thus the former 7 AVPs exhibit high stability than the later 11 AVP structures investigated. The other 13 AVPs (1VM5, 1LFC, 1RPB, 1F0F, 1G89, 1BH4, 1E4S, 1ZFU, 1KAL, 1K48, 1R1F, 2GJ0 and 2B5B) showed positive energy values, thereby, meaning that they have considerably low stability. We have also observed that the AVP (1ZMP) exhibits a total energy of **-6833.40** kcal/mol which we consider to be highly stable as compared with other AVPs by total energycomparison.

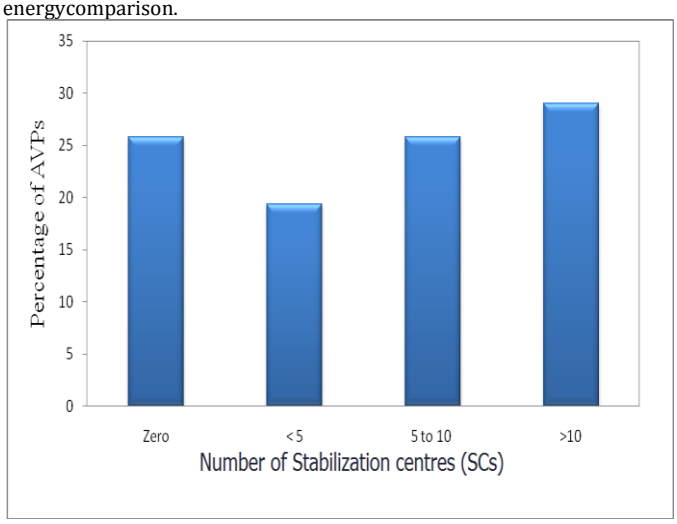

Fig 2: Stabilization centers (SCs) in Anti-viral **peptides (AVPs).**

Energetic analysis of a cation-π interaction in AVPs

Within a protein, cation-π interactions can occur between the cationic sidechains of either lysine (Lys, K) or arginine (Arg, R) and the aromatic sidechains of phenylalanine (Phe, F), tyrosine (Tyr, Y) or tryptophan (Trp, W). Cation-π interactions have recently been observed in many biological systems including proteins. We used the web-based version of the CaPTURE program[39] (http://capture.caltech.edu) to detect any cation-π interactions and to determine their strength(s). The program detected significant cation–π interaction in only eight peptides (3GNY, 3HJ2, 1DFN, 1ZMP, 1ZMQ, 1RKK, 2ERI and 2GJ0) out of 31 AVPs investigated in this work. The result is shown in Table 3. Out of 8 AVPs, two PDB ID's, namely 3GNY and 1ZMP having 2 significant interactions and the other six PDB ID's, namely 3HJ2, 1DFN, 1ZMQ, 1RKK, 2ERI and 2GJ0 having only one significant cation-π interactions. It was interesting to note that 1ZMP also shown lower energy of -6833.40 kcal/mol as compared to all the other AVPs investigated in this work. Although 1ZMP exhibits 2 significant cation-π interactions, the total cation-π interaction strength is only of -6.44 kcal/mol (electrostatic energy component -3.03 kcal/mol and van der Waals energy component -3.41 kcal/mol), almost similar magnitude to that of other 7 AVPs investigated in this work. Hence, a decision could not be made on the comparison of the stability of these AVPs with the results obtained so far. Consequently we decided to further continue to investigate these four AVPs (3GNY, 3HJ2, 1DFN, 1ZMP, 1ZMQ, 1RKK, 2ERI and 2GJ0) through intra-molecular interactions and instability index which gave a better insight into the stability of these AVPs.

Non-Covalent and Non-Canonical Interactions in the structural stability of AVPs

The details of intramolecular interactions in the structure of AVPs investigated are shown in Table 4 and 5. Although the three-dimensional structure of a peptide/protein is stabilized by non covalent interactions such as hydrogen bonds, salt bridge and di-sulfide bridge, the non canonical interactions such as C–H...π, N-H...π and C-H...O interaction also contributes to the structural stability of AMPs[57-58]. The Pymol view of N-H...π and C-H...O interaction in the structure of "Human α-defensin 5 (PDB ID: 1ZMP)" is shown in Fig 3. Even though the non conventional interactions are not only comparable in strength to non-covalent interactions but cumulatively can afford a certain degree of stability to the AMPs structure. Therefore we decided to investigate both non covalent and non canonical interactions in the structural stability of AVPs. This analysis portrays that AVPs such as 1ZMP and 1ZMQ have high number of intramolecular interactions (i.e. 91 and 88 respectively) as compared to the other six AVPs (3GNY, 3HJ2, 1DFN, 1RKK, 2ERI and 2GJ0) investigated in this work. Hence we have decided to address the instability index value for these two AVPs (1ZMP and 1ZMQ), which is helpful for the identification of potential candidate among the pool of AVPs investigated in this work.

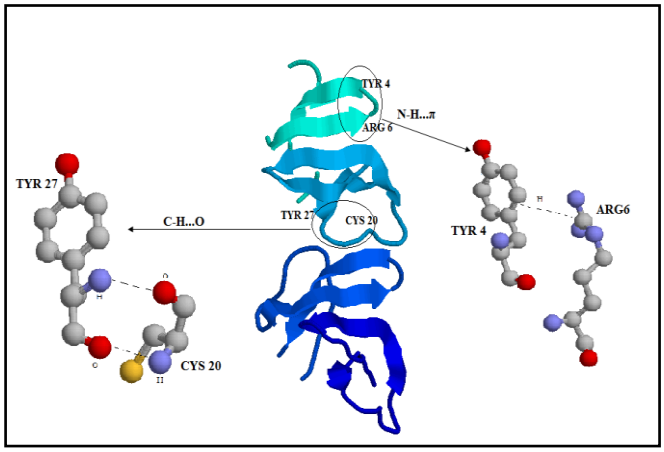

Fig 3: Pymol view of non-canonical interaction in the structure of human α-defensin 5.

Overall Stability by Instability Index

Further interrogation based on Instability index[56] for the two AVPs such as 1ZMP and1ZMQ showed that 1ZMP has the instability index of 13.79 when compared to 1ZMQ with an instability index of 75.96 (Table 5). Thus 1ZMP has a lesser instability index. It is known that lower is the instability index more is the stability of the peptide.

DISCUSSION

It is well known that 1ZMP is Human α-defensin 5 (HD5) frequently referred to as intestinal defensin, since they are most abundant in the epithelia of the Paneth cells[59-60]. The comparable activity against three pathogens was also observed for HD5[61] (PDB ID: 1ZMP). This defensin appeared to be more potent than HNP1 or HNP4 against *S. aureus*, whereas anti-yeast activity was quite low. Curiously, HD6 (PDB ID: 1ZMQ) was practically inactive. The only modest response to HD6 (PDB ID: 1ZMQ) was observed for *E. faecium.* In contrast, HD5 (PDB ID: 1ZMP) was very potent against this digestive tract bacterium, and the complete inhibition was observed at concentrations of defensin <1 mg/ml. HD5 (PDB ID: 1ZMP) kills Gram-positive *S. aureus* and *E. faecium* more efficiently than Gram-negative *E. coli* or *P. aeruginosa.* Neither of α-defensins efficiently kills yeast. HD6 (PDB ID: 1ZMQ) showed practically no antimicrobial activity in the experimental study[61].

The difference in biological activity is mainly due to the significant conformational differences in the specific sections (β-hairpin) of the molecules HD5 and HD6[61]. That is a potential role of dimerization of the peptide monomer as a possible reason for their activity. The dimer stabilizing interactions are more extensive in HD5 as compared to HD6. The network of intermonomer contacts observed for HD6 is different than HD5 i.e. the arrangement of the hydrogen bonds between two β2 strands is different thus affecting the dimerization capacity of HD6 which in turn affects its activity. Recently another group also reported similar results for all six human α-defensin[62]. Also, the unusually

high activity of HD5 (PDB ID: 1ZMP) against human papilloma viruses was recently reported[63]. Hence by this study, we can without any ambiguity claim that 1ZMP, Human α-defensin 5 (HD5) could be considered as a potential peptide to be a template for designing therapeutic peptide drug against viral based disease concern. The study of Shen et al. [64] reported the first ever recorded bacterial resistance to synthetic as well as natural AMPs in *Vibrio parahaemolyticus* suggesting that the resistance development may be through the up regulation of the multidrug efflux transporter, effective repair ofdamaged membranes and prevention of cellular penetration of AMPs. Recent approaches suggest that the combined use of lower concentrations of conventional antibiotics and AMPs should be of immense importance to overcome resistance development.

Table 2: Total energy and total number of cation-π interactions in the AVPs data set.

PDB Code	Total Energy (kcal/mol)	Total number of cation-π interactions	PDB Code	Total Energy (kcal/mol)	Total number of cation-π interactions
1VM5	467.228	NA	2ERI	-397.957	1
1LFC	182.758	NA	1KJ6	-1563.777	NA
1RPB	953.253	NA	2K6O	-830.428	NA
1F0F	783.790	NA	1ZRV	-866.647	NA
2MLT	-166.410	NA	1HVZ	-988.984	NA
1G89	529.737	NA	1E4S	343.517	NA
3GNY	-2727.606	3	1ZFU	124.724	NA
3HJ2	-1697.133	1	2FBS	-211.752	NA
1DFN	-1543.166	1	1KAL	791.811	NA
1ZMM	-5226.609	NA	1K48	824.190	NA
1ZMP	-6833.40	4	1R1F	236.825	NA
1ZMQ	-2415.927	4	1ZA8	-82.183	NA
1PG1	-632.074	NA	2GJ0	402.290	1
1RKK	-172.173	1	2ATG	-534.549	NA
1WO1	-364.724	NA	2B5B	1321.830	NA
1BH4	444.222	NA			

NA: Not available

Table 3: Energetic analysis of cation-π interactions detected in the structure of AVPs.

PDB Code	Cation-π interacting residue pairs		E_{es} (kcl/mol)	E_{vdw} (kcal /mol)	Total energy (kcal/mol)
3GNY	Arg (5)	Tyr (3)	-2.28	-1.8	
	Arg (14)	Tyr (16)	-3.7	-1.92	-9.7
3HJ2	Arg (8)	Tyr (6)	-2.63	-1.66	-4.29
1DFN	Arg (6)	Tyr (4)	-2.51	-1.87	-4.38
1ZMP	Arg (6)	Tyr (4)	-1.64	-1.54	
	Arg (25)	Tyr (27)	-1.39	-1.87	-6.44
1ZMQ	Arg (7)	Tyr (11)	-4.02	-4.29	-8.31
1RKK	Arg (2)	Trp (3)	-3.08	-1.56	-4.64
2ERI	Lys (20)	Tyr (25)	-2.50	-0.78	-3.28
2GJ0	Lys (14)	Tyr (16)	-4.92	-1.06	-5.98

Table 4: Non-covalent interactions in the structure of AVPs.

PDB Code	Hydrogen Bond	Hydrophobic intraction	Disulphide bridge	Salt bridge	Total number of Interactions
3GNY	**21**	28	12	3	64
3HJ2	22	26	12	1	61
1DFN	20	24	12	3	59
1ZMP	44	18	20	4	86
1ZMQ	46	32	24	5	107
1RKK	2	8	4	4	18
2ERI	9	16	6	4	35
2GJ0	9	14	6	3	32

Table 5: Non-canonical interactions in the structure of AVPs.

PDB Code	C-H...π interaction analysis	N-H...π interaction analysis	C-H...O interaction analysis	Total number of NCI	Instability index
3GNY	2	4	12	18	
3HJ2	NA	NA	13	13	
1DFN	8	4	10	22	
1ZMP	NA	4	19	23	13.79
1ZMQ	4	NA	9	13	75.96
1RKK	NA	NA	1	1	
2ERI	NA	NA	3	3	
2GJ0	NA	NA	4	4	

NA: Not available

Despite recent advances in medical diagnostics and treatment methodologies, viral damage remains a major source of morbidity and mortality throughout the world. Several attempts have been made over recent years to advance novel broad-spectrum anti-viral peptides into clinical use with limited success and the reasons for this failure are certainly diverse but the key unresolved issues regarding stability of the

peptide is one of the major causes of the lack of systemic application, towards which peptide therapeutic application holds the most potential. This issue could be addressed only by designing synthetic peptides with the aid of appropriate template molecule. Thus, the use of computational methods in the identification of AMPs stability is becoming an important and promising approach for improving the synthetic AMPs development. Based on the energetic values found in our AVPs study in addition to the SCide, non-covalent interactions,non-canonical interactions and instability index, scrutiny agrees that Human α-defensin 5 (HD5) should be the template for designing the therapeutic peptide drug against viral pathogens as it was also well supported by experimental study elsewhere. The ingenuity and success of the discovery efforts discussed above bode well for the future prospects of finding new therapeutics which could results into massive reductions in therapeutics development time, which would provide us a hefty head-start against our antibiotic-resistant viral adversaries.

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