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In Silico Modelling of Engineered LL-37

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Abstract

The ability to predict three dimension conformations and energies of small peptides is quite crucial in protein engineering based drug designing. The current study is aimed to engineer LL-37 peptide by Mechanism and Empiricism based protein engineering. Template LL-37 was obtained from PDB database and used for similar sequences retrieving by Blast P. 62 sequences obtained were used for multiple sequence alignment and protein engineering of LL-37. Total 200 variable peptides developed were used for model development by automated mode Swiss model and PEPFOLD server. Models obtained were energy minimized and used for evaluation by PROCHECK and Q-Mean servers. Based on the Ramachandran Plot and Q-Mean Z-score values 200th variant was found to be highly stable compared with wild and remaining variants. The predicted 3D model can be used for various drug design studies in laboratory.

Keywords: Protein engineering, LL-37, Cathelicidin, Multiple sequence alignment, Ramachandran Plot and Z-score.

1. INTRODUCTION

Recurrent use of antibiotics leads to development of antibiotic resistant strains like methicillin resistant *staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococcus* (VRE) and carbapenem-resistant *Enterobacteriaceae* (CRE). Antibiotic resistance is a threatening alarm for the invention of novel treatment methods. Antimicrobial peptides (AMPs) are alternatives for conventional antibiotics (1) having

broad spectrum of antimicrobial activity against antibiotic resistant bacteria. Antimicrobial peptides are produced by unicellular to multicellular organisms. Antimicrobial peptides are released as a part of innate immune system of first line of defence. AMPs have promising role for the treatment of various skin infections (2). Pexiganan is engineered for increased efficiency against many bacteria. In vitro, pexiganan showed significant antimicrobial activity against clinical isolate cultures from infected Diabetic Foot Ulcers (3).

Cathelicidins and Defencins are most common group of AMPs used in humans. Human cationic antimicrobial protein ~18kDa (hCAP-18) contains highly conserved cathelin N-terminal domain and less conserved C-terminal antimicrobial peptide. C –terminal antimicrobial peptide contains 37 amino acids and initially starting with two leucine residues, denoted as LL-37(4). LL-37 has potent antimicrobial, angiogenic; wound healing and apoptotic activities (5). In the present study, an attempt was made to develop variants of LL-37 for improved activities using protein engineering and 3D structure development to the variants obtained.

2. METHODOLOGY

2.1. Retrieval of sequence:

Amino acid sequence of LL-37 an antimicrobial agent was obtained from RCSB PDB database (http://www.rcsb.org/pdb/explore/explore.do?structureId=2k6o) (6). FASTA sequence was used for search in non- redundant (nr) data base at NCBI using BLASTp algorithm.

2.2. Multiple sequence alignment:

LL-37 and its similar amino acid sequences were used in Multiple Sequence Alignment (MSA) through Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) (7). Template and target FASTA sequences were submitted in the work space area with default parameters. Aligned sequences were inspected and manually the gaps and insertions were removed. It is designed to align the sequences from high to least similarity of all sequences.

2.3. Empiricism-based Protein engineering:

Non-conserved amino acids were used for protein engineering. Early modification i.e close related sequences were used for modification initially and delayed modifications later. Conserved region amino acids were not incorporated in further construction of engineered peptides. In combinatorial based protein engineering main concentration on the similarities and dissimilarities between the different groups arranged phylogenetically having minimum distance from different depositories like PDB Database, UniPort Database and SWISS PORT database etc., Phylogenetic modifications were found in the multiple aligned sequences used for development of 100 LL-37 variants.

2.4. Mechanism-based protein engineering :

As lysine is prone for protease activity on it, it has been replaced by arginine in various positions (8). Lysine is replaced with Arginine which leads to increase in hydrogen bonds formation all over the structure of proteins. The energy values were reduced and stability increased in the protein (9).

2.5. Modeling of LL-37 variants:

Homology modelling of engineered peptide was done using SWISS-MODEL (10) for peptides above 30 amino acids and PEP-FOLD (11) work space for short peptides of 9 to 30 amino acids. In SWISS-MODEL, Template identification was done for each engineered sequence in the automated model development. Once the template was identified modelling carried out using same server in alignment mode. In order to get optimized alignments, 3 optimized models were obtained for above 30 amino acid peptides by automatic modelling mode of SWISS model. PEP-FOLD server used to develop 1-5 3D conformations of peptides from 9 to 30 amino acids length.

2.6. Validation of model:

The model was further refined with energy minimization with Swiss PDB viewer (12). The models were further validated using Q-mean server (13) and PROCHECK analysis (http://www.ebi.ac.uk/thornton-srv/software/PROCHECK/) (14).

3. RESULTS:

3.1. Sequence collection:

LL-37 is a host defense peptide normally present in higher animals. BLAST in the NCBI Blast P of LL-37 (PDB ID: 2K6O), resulted 62 similar sequence. The following are the retrieved sequences and their IDs: XP_006733054.1, XP_016048219.1, 7533667.2, 7446282.1, XP_004325464.1, XP_006165951.1, NP_001003359.1, 15345101.1, XP_004368778.1, XP_012590677.1, XP_017524803.1, 17524805.1, 6868849.1, XP_006893092.1, 8572297.1, XP_007949964.1, 8047795.1, XP_004449765.1, 4648793.1, 5075137.1, Q1KLY3.1, XP_004034110.1, pdb|2LMF|A, XP_002813845.1, Q1KLX2.1, pdb|2K6O|A, CAA86115.1, NP_001065283.1, P49913.1, XP_003818474.1, NP_004336.3, pdb|2FCG|F, pdb|2L5M|A, Q1KLX8.1, XP_003257051.1, ABE96625.1, Q1KLX9.1, Q1KLY0.1, Q1KLY2.1, Q1KLX4.1, Q1KLX5.1, XP_017711138.1, XP_011794578.1, XP_010373021.1, XP_007982284.1, Q1KLY6.1, NP_001028681.1, XP_011836404.1, XP_005547060.1, XP_011737492.1, Q1KLX7.1, Q1KLX3.1, XP_003894539.1, XP_017371350.1, Q1KLY5.1, XP_017820371.1,Q1KLY4.1, Q1KLY7.1, Q1KLY8.1, XP_012329235.1, Q1KLX0.1, XP_003926342.1. The FASTA sequences were retrieved and used for further analysis.

3.2. Multiple sequence alignment:

Multiple sequence alignment of 62 peptides using Clustal Omega revealed conservation of three amino acids at 31, 32, 33 among 60 sequences and followed by 22, 23, 24, 25

and 26, 5 amino acids conserved among 54 sequences**.** 18, 19, 20 amino acids are very less conserved in the sequences studied (Fig: 1).
The wild type of LL-37 peptide was modified with two different protein engineering approaches.

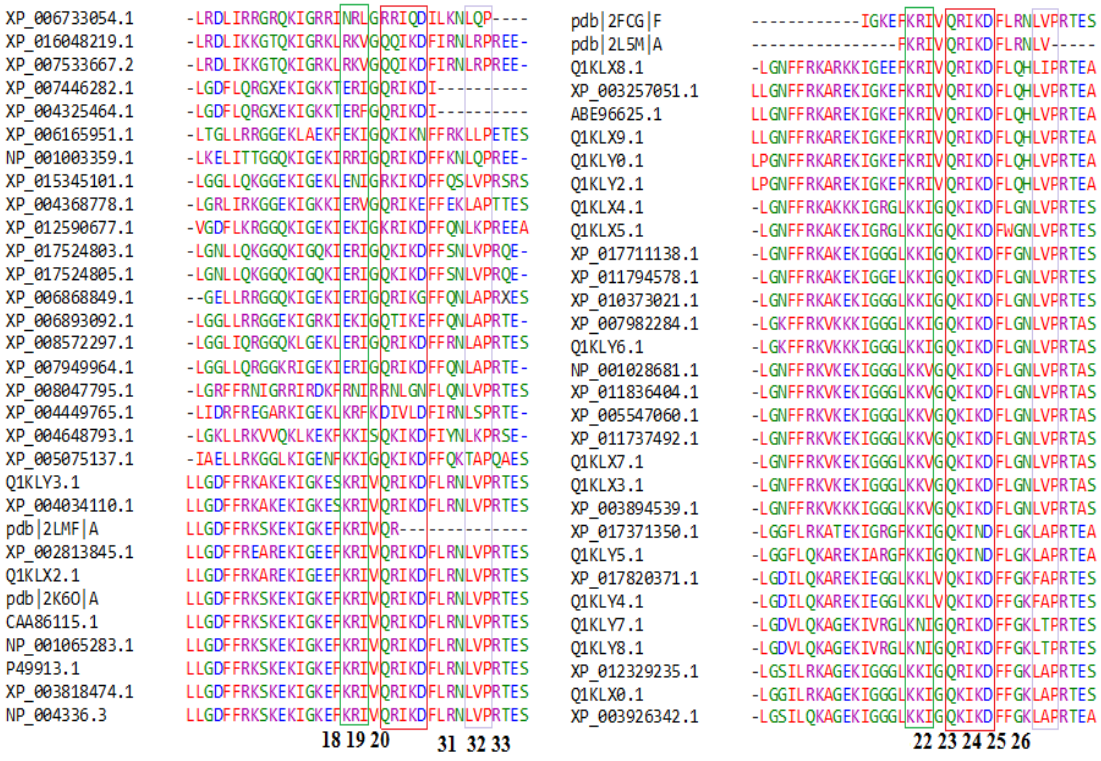


Figure 1: Multiple sequence alignment of LL-37 and similar sequences.

3.3. Empiricism based Protein engineering:

Empiricism based analysis the revealed most conserved sequence present at N-terminus of peptides. Based upon the phylogenetic analysis, initial modified leucine with Proline 2Leu- Pro, Asparagine with Aspartic acid 4Asn-Asp, Lysine with Glutamic acid modified in 8 and 15 positions Lys-Glu, Alanine with Serine 9Ala-Ser, Lysine with Arginine 10Lys-Arg, Phenylalanine with Serine17Phe-Ser, Glutamine replaced with Arginine 29Gln-Arg, Histidine with Arginine 30His-Arg, Alanine with Serine 37Ala-Ser were implemented to developed100 variable peptides of LL-37.

3.4. Mechanism based protein engineering:

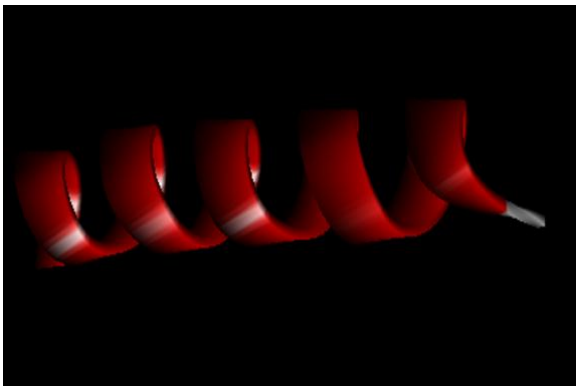
Mechanism based protein engineering resulted by replacing Lysine with Arginine in 8^{th}, 10^{th} ,12^{th} , 15^{th} and 18^{th} positions in the wild LL-37 peptide and 100 variants developed.

3.5. 3D structures development and validation:

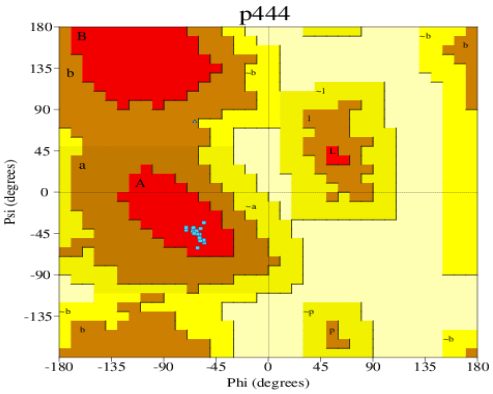
Q-Mean server was used for determining model reliability and PROCHECK for calculating Ramachandran plot. PROCHECK Ramachandran Plot of LL-37 V-200

model had highest number of residues in the most favoured regions (100.0%) compared to the wild LL-37 (93.8%) (Fig: 2 and 3).

Out of 200 LL-37 variant, 20 variants were resulted highly reliable 3D structures, Ramachandran plots and Z-score values. The 20 sequences details were given table 1. The QMEAN score for LL-37 V-200 was the lowest (0.14) compared with the existing model LL-37 (0.638).



(a)

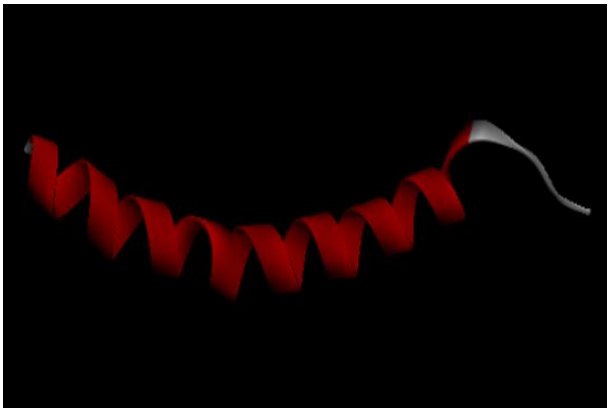


(b)

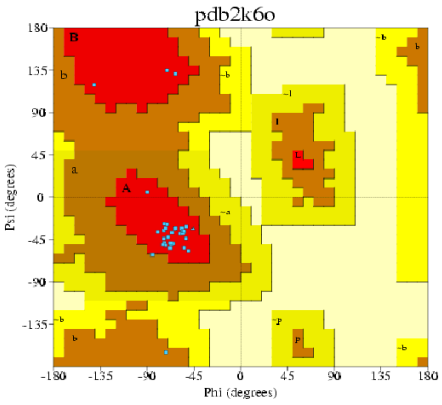
		residues	%-tage
Most favoured regions	[A,B,L]	18	100.0%
Additional allowed regions	[a,b,l,p]	0	0.0%
Generously allowed regions	[~a,~b,~l,~p]	0	0.0%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		18	100.0%
End-residues (excl. Gly and Pro)		1	
Glycine residues		2	
Proline residues		0	
Total number of residues		21	

(c)

Figure 2: (a) (b) and (c): LL-37 200 variant and its Ramachandran plot



(d)



(e)

		No. of residues	%-tage
Most favoured regions	[A,B,L]	30	93.8%
Additional allowed regions	[a,b,l,p]	2	6.2%
Generously allowed regions	[~a,~b,~l,~p]	0	0.0%
Disallowed regions	[XX]	0	0.0%
Non-glycine and non-proline residues		32	100.0%
End-residues (excl. Gly and Pro)		2	
Glycine residues		2	
Proline residues		1	
Total number of residues		37	

(f)

Figure 3: (d) (e) and (f): LL-37 and its Ramachandran plot

Table 1: Z-score values of LL-37 and LL-37 peptide variants

S.No	LL37V																																		Z-score
1	1****1	L	L	G	D	F	L	R	K	S	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S	37	0.36
2	12	L	L	G	D	F	I	R	K	S	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S	37	0.33
3	17	L	L	G	D	F	F	R	K	A	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S	37	0.34
4	34	L	L	G	D	F	F	R	K	S	K	E	K	I	G	K	E	L	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S	37	0.4
5	35	L	L	G	D	F	F	R	K	S	K	E	K	I	G	K	E	S	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S	37	0.62
6	48	L	L	G	D	F	F	R	K	S	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	F	R	N	L	V	P	R	T	E	S	37	0.39
7	49	L	L	G	D	F	F	R	K	S	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	W	R	N	L	V	P	R	T	E	S	37	0.39
8	56	L	L	G	D	F	F	R	K	S	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	I	P	R	T	E	S	37	0.33
9	58	L	L	G	D	F	F	R	K	S	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	T	P	R	T	E	S	37	0.49
10	66	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	–	–	–	–	–	15	0.26
11	69	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	F	K	R	I	V	Q	R	I	K	D	F	L	R	H	L	V	–	–	–	–	–	16	0.19
12	70	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	I	–	–	–	–	–	16	0.88
13	103	L	p	G	D	F	F	R	K	S	K	E	K	I	G	K	E	S	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S	37	0.45
14	158	L	p	G	N	F	F	R	K	S	K	E	K	I	G	K	E	S	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S	37	0.52

Table 1 Countinued…

S.No	LL37V																																			Z-score
15	161	L	p	G	N	F	F	R	K	S	K	E	K	I	G	K	E	F	K	K	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S	37	0.45
16	189	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	F	K	N	I	V	Q	R	I	K	D	F	L	R	N	L	V	S	–	–	–	–	18	0.36
17	191	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	F	K	R	I	G	Q	R	I	K	D	F	L	R	N	L	V	S	–	–	–	–	18	0.44
18	192	–	–	–	–	–	–	–	–	–	–	–	–	–	G	–	–	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	S	–	–	–	–	18	0.54
19	200	–	–	–	–	–	–	–	–	–	–	–	–	G	G	H	D	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	N	–	–	–	–	21	0.14
20	LL37 org	L	L	G	D	F	F	R	K	S	K	E	K	I	G	K	E	F	K	R	I	V	Q	R	I	K	D	F	L	R	N	L	V	P	R	T	E	S	37	0.63

4. DISCUSSION

In this modern era of highly evolving microbial variants and failure of many antibiotics, drug designing by protein engineering has become one of the important means (15 and 16). LL-37 a host defence cathelicidin, produced physiologically in higher animals and humans has been implicated in many studies for its antimicrobial activity and its conserved structure over generations (17).

Research work was carried out on LL-37's antimicrobial activity against different kind of pathogenic micro-organisms (18). On the other hand, LL-37 also enhances angiogenesis activity (19). Protein engineering of antimicrobial peptides by one or two amino acid modification was found to improve its antimicrobial activity at larger extent (16). Several investigations (20 and 21) concluded that short peptides have more antimicrobial activity than larger one. The present study establishes that the shorter peptides have highly stable structures and also high possible antimicrobial activity. Most of protein engineered work carried out so for is based on rational engineering (16). In the present study we have combined rational and empiricism based protein engineering to obtain.

In the current study, combined of both Empiricism and mechanism based protein engineering for development of 200 LL-37 variants. From these 200, variants having more than 30 amino acid peptides 3D structures were developed using SWISS MODEL work space. 9-30 amino acid peptide structures were modelled through PEP-FOLD server. In rational based protein engineering, 3D structures of both anti-angiogenesis peptides buPRL-derived peptide (AQGKGFITMALNSC) and the scrambled peptide (TASQGFINACGMLK)) were obtained through PEP-FOLD server and Bradykinin I receptor structure was developed in SWISS MODEL server. The anti-angiogenesis 14 amino acids containing peptides as well as receptor structures were further validated by PDBsum and PROCHECK (11).

The accuracy and stereo chemical features of engineered LL-37 peptides were calculated with PROCHECK and Q- mean server. 3D structure of Hsp 90 protein was generated, validated with PROCHECK and Q-mean server and successfully submitted to PMDB (18). The engineered LL-37 peptides were validated through Q-mean server. Q-Mean Z-score value gives the degree of native ness of the structure. The average Z-score of high resolution model is "Zero" and predicted global model reliability lies

between 0-1. Q MEAN server based 'Z' score is highly reliable by relating it to reference structure solved by X-ray chromatography (18). Q MEAN Z score of 0-1 is stable and v200 Z score is 0.14.

Protein engineered variants based on empiricism and mechanism (100 each) were developed. The LL-37 v 200 obtained by empiricism based engineered, 3D modelled through PEP FOLD and validated by Q-mean server and PROCHECK was found to be stable and potent antimicrobial agent for drug development.

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