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ORIGINAL PAPER

Molecular modeling of four Dermaseptin-related peptides of the gliding tree frog *Agalychnis spurrelli*

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Abstract

In this research, we present a preliminary computational study of four Dermaseptin-related peptides from the skin exudate of the gliding tree frog *Agalychnis spurrelli*. Experimentally, the amino acid sequence of these peptides was elucidated through molecular cloning and tandem mass spectrometry and synthetic peptides were assayed against *E. coli*, *S. aureus*, and *C. albicans* to determine their antimicrobial properties. With the sequences on hand, a computational study of the structures was carried out, obtaining their physicochemical properties, secondary structure, and their similarity to other known peptides. A molecular docking study of these peptides was also performed against cell membrane and several enzymes are known to be vital for the organisms. Results showed that Dermaseptin-related peptides are α-helical cationic peptides with an isoelectric point above 9.70 and a positive charge of physiological pH. Introducing theses peptides in a database, it was determined that their identity compared with known peptides range from 36 to 82% meaning these four Dermaseptins are novel peptides. This preliminary study of molecular docking suggests the mechanism of action of this peptide is not given by the inhibition of essential enzymatic pathways, but by cell lysis.

Keywords *Agalychnis spurrelli* · Antimicrobial peptides · Dermaseptins · Molecular docking

Introduction

The skin secretions of several frogs contain plenty number of compounds with biological activity, which are very interesting for designing and developing possible new drugs [1]. These molecules include peptides that are synthetized and stored in granular glands of the frog skin, and usually have broad spectrum of antimicrobial activity against bacteria, fungi, and protozoa. These molecules are part of the animal's defense mechanism and innate immunity system [1–5].

In the last decade, more than 300 bioactive peptides with different pharmacological properties have been isolated from frog skin secretions, presenting an excellent source of therapeutic compounds [2]. Using new technology, it was possible to obtain 76 individual peptides from the skin of the Russian brown frog [6].

The primary function of these peptides is to protect frogs against pathogens. Studies have shown activity against viruses, cancer cells, antitumor, antidiabetic, antioxidant, enzyme inhibition, and chemostatic effects that make them interesting for the discovery of new molecules in the fight against antibiotic-resistant microorganisms [3, 7]. In addition, recent

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studies have identified a number of these antimicrobial peptides that can modulate inflammatory response [2]. Currently, the use of these peptides is limited due to their unknown toxicity towards mammalian cells such as erythrocytes [1].

Peptides obtained from frog skin vary in size, from 8 to 48 amino acids long. A comparison in their sequences reveals the absence of conserved domains associated with their biological activity. However, with few exceptions, these peptides are cationic with a molecular charge between + 2 and + 6 at pH 7 due to the presence of multiple basic amino acids like lysine. In addition, they contain at least 50% hydrophobic amino acids from which leucine and isoleucine are the most abundant. Magnetic resonance imaging (MRI) and circular dichroism studies showed that these peptides lack a defined secondary structure in aqueous solutions but tend to form an alpha helix in a phospholipid environment or in solvents that mimic the membrane [1].

There is no single mechanism by which peptides cause cell death. Normally, their action does not involve interaction with a specific receptor. Rather, it is a non-specific interaction with the bacteria's cell membrane that results in its permeabilization and disintegration [1, 7]. These interactions, often complex, can lead to the peptides sticking to the membrane, membrane permeability, rupture, and cell lysis. This is one of the reasons these peptides are active against antibiotic-resistant bacteria [1]. Spectroscopic and computational analyses suggest that structural flexibility of the N- and C-terminals plays a crucial role in determining the peptide's membrane interaction mode [8].

Agalychnis spurrelli, commonly called gliding tree frog, is an amphibian species that belongs to the order Anura and *Hylidae* family. This frog lives in Costa Rica, Panama, Colombia, and Ecuador. In Ecuador, they can be found in the provinces of Esmeraldas, Los Rios, Manabí, Carchi, and Pichincha. Its population can be very abundant. Studies conducted in Esmeraldas estimate a population of over 2000 adults in 2400 m^2 [9]. They are nocturnal species that live in rainforests and close to bodies of water where they reproduce [9–12].

The aim of this work was to computationally model the interaction of these peptides with the microorganisms of interest, through different methods and software such as molecular coupling (docking), in order to establish a preliminary mechanism of action of these peptides.

Methodology

The frog *Agalychnis spurrelli* was collected in the Province of Esmeraldas. The specimens collected were transported alive under conditions of adequate humidity and temperature, and then kept in individual terrariums in a *bioterio*.

Skin secretions from specimens collected from *Agalychnis spurrelli* were obtained by dorsal massage and the exudate was washed with distilled water to lyophilize it and keep it at − 20 °C until its use.

The process of molecular cloning begins with the extraction of mRNA for which an mRNA extraction kit was used from 1 mg of lyophilized secretion. The 3′CDS and 5′CDS primers and the reverse transcriptase enzyme were used to construct a cDNA library, which was stored at − 20 °C. Rapid amplification of final cDNA fragments (RACE PCR) was performed. The amplification was verified by means of electrophoresis.

The RACE PCR products were purified by column and dehydrated in the vacuum concentrator. These products were resuspended in 2–8 μl of bidistilled water. A ligation reaction was prepared using the T-Easy vector, the PCR product, and the Ligase T4. The reaction was left for 1 h at room temperature and then at 4 °C overnight. Then the recombinant vector was introduced into competent *E. coli* JM109 cells by thermal shock. Transformed cells were cultured on LB agar supplemented with ampicillin, IPTG, and XGal. The white and blue selection was used to recognize the recombinant colonies.

Once the phenotype of the colonies was confirmed, the plasmid DNA was extracted using the simple boiling technique. Subsequently, the recombinant segment was amplified by PCR using M13R and M13F primers bordering the multiple cloning site of the T-Easy vector.

The DNA sequences obtained were analyzed by bioinformatics techniques and also modeled. The peptides selected according to the bioinformatic analyses were chemically synthesized.

In order to determine the synthetic peptides' bioactivity, the minimal inhibitory concentration (MIC) was determined against ATCC bacteria strains of *E. coli*, *S. aureus*, and yeast *C. albicans*. Serial dilutions of each peptide in dimethylsulphoxide (DMSO) were made to obtain concentrations of 512, 256, 128, 64, 32, 16, 8, 4, 2, 1×10^2 mg/L. Each microorganism in log phase was diluted to obtain the equivalent of 1×10^6 colony forming units (CFU)/mL for the bacteria and 1×10^5 CFU/mL for the yeast. Later, 2 μL of each peptide dilution was transferred to a 96-well sterile plate and 198 μL of the microorganism culture was added. As controls, 2 μL of DMSO was included instead of peptide and 200 μL of Mueller-Hinton Broth in another well. Seven replicates per peptide concentration were performed on each plate and the experiment was repeated 3 times in order to confirm the results. Plates were incubated overnight at 37 °C. After 18–22 h of microorganisms growing, the plates were read at λ = 550 nm in an ELISA plate reader.

Different computational techniques were used to study the four Dermaseptin-related peptides from the skin exudate of the *Agalychnis spurrelli* frog, including different software, servers, and web pages. The physicochemical properties of

the peptides were predicted using ExPasy [13], Pepcalc [14], Biosyn [15], JPred 4 [16], and PSIPred [17]. Comparisons of the four sequences with other peptides from different amphibian species were performed using Uniprot database (https://www.uniprot.org/). The three-dimensional structure of the peptides was obtained using Pymol [18]. The peptide structure was relaxed in a first step using statistical mechanics with the MM2 force field implemented in the ChemDraw software [19] and optimized by quantum chemical calculations using ONIOM HF method and a 6-31G basis set implemented in Gaussian 09 package of programs [20]. The three-dimensional structures of the microorganism enzymes were downloaded from the RCSB Protein Data Bank [21]. AutoDock Tools was used to eliminate waste, identify the area of the enzymes where the calculations are going to be carried out, and obtain all the structures in the appropriate format (.pdbqt). With the optimized peptides, a molecular docking study between them and the enzymes was carried out using Autodock VINA [22]. The three-dimensional structures of membranes were downloaded from the RCSB Protein Data Bank [21]. Furthermore, the interaction of these peptides with mammalian and bacterial cell membrane and with molecules known to be part of bacterial cell membrane was performed to obtain more information about their mechanism of action. Peptides used in this part of the research incorporated full side chain flexibility in the whole molecule. All PDB entries of the structures used in this research can be found in the supporting information (Table S1).

Results and discussion

Four Dermaseptin-related peptides (DRP-SP2 to DRP-SP5) were characterized through molecular cloning and tandem mass spectrometry from the skin exudate of *Agalychnis spurrelli*. The four sequences were deposited in Genbank with accession numbers MK532480 to MK532483 (Table 1).

With the sequence on hand, these peptides were synthetically produced and tested experimentally against three microorganisms *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. The experimental results are presented in Table 2.

The experimental results show that the DRP-SP2 has antimicrobial properties against Gram-negative bacteria (*E. coli*), Gram-positive bacteria (*S. aureus*), and fungi (*C. albicans*). The DRP-SP3 and DRP-SP4 peptides have antimicrobial activity against *E. coli* and *S. aureus* while their activity against *C. albicans* is undefined. DRP-SP5 showed activity against *E. coli* and *C. albicans*, but its activity against *S. aureus* could not be defined.

Table 2 Experimental bioactivity of Dermaseptin-related peptides against microorganisms

Peptide	Microorganism	Strain	Bioactivity	MIC*
DRP-SP2	*E. coli*	ATCC 25922	YES	8
	S. aureus	ATCC 25923	YES	8
	C. albicans	Clinical isolated	YES	32
DRP-SP3	*E. coli*	ATCC 25922	YES	128
	S. aureus	ATCC 25923	YES	512
	C. albicans	Clinical isolated	Undefined	> 512
DRP-SP4	*E. coli*	ATCC 25922	YES	128
	S. aureus	ATCC 25923	YES	512
	C. albicans	Clinical isolated	Undefined	> 512
DRP-SP5	*E. coli*	ATCC 25922	YES	256
	S. aureus	ATCC 25923	Undefined	> 512
	C. albicans	Clinical isolated	YES	1054

*Minimum inhibitory concentration (mg/L)

Once in vitro antimicrobial activity was determined, the peptides were modeled and characterized by computational techniques. First, physicochemical properties of the four structures were calculated as shown in Table 3.

Among their predicted properties, these Dermaseptin-related peptides are short-chain peptides (from 22 to 28 amino acids long) with two to four basic amino acids and one negatively charged amino acid in their structure. This confers a basic characteristic reflected in their isoelectric point (9.71 to 11.48) and their net charge (greater than zero at pH 7).

The extinction coefficient is defined as the capacity of a certain substance to absorb light at a certain wavelength by molar concentration [23]. For peptides, the wavelength studied is 280 nm. This measure closely related to the composition of the peptide, since only few amino acids have the capacity of absorbing electromagnetic radiation at this wavelength. Those amino acids are the ones with an aromatic ring in their structure such as tyrosine, tryptophan, and phenylalanine. Tryptophan is the one with the biggest molar absorptivity. All the Dermaseptin-related peptides studied have one or more of these amino acids in their sequence, producing a signal when they are analyzed by UV spectroscopy.

Table 1 Amino acid sequence and length of the four studied Dermaseptin-related peptides (DRP)

Name	Acronym	Sequence	Length
Dermaseptin-SP2	DRP-SP2	ASWKVFLKNIGKAAGKAVLNSVTDMVNQ	28 aa
Dermaseptin-SP3	DRP-SP3	SLWSSIKDMAAAAGRAALNAVNGIVNP	27 aa
Dermaseptin-SP4	DRP-SP4	SLWSSIKDMAAAAGRAALNAVNGILNP	27 aa
Dermaseptin-SP5	DRP-SP5	SLRSSIKDMAAAAGRAALNAVNGIVNP	27 aa

Table 3 Physicochemical properties of the four Dermaseptin-related peptides isolated from *Agalychnis spurrelli*

Parameter	DRP-SP2 [24]	DRP-SP3	DRP-SP4	DRP-SP5
Length	28	27	27	27
Molecular weight	2990.51	2698.09	2712.12	2668.07
pI	10.80	9.71	9.71	11.48
Formula	$C_{134}H_{221}N_{37}O_{38}S_1$	$C_{117}H_{193}N_{35}O_{36}S_1$	$C_{118}H_{195}N_{35}O_{36}S_1$	$C_{112}H_{195}N_{37}O_{36}S_1$
# of atoms	431	382	385	381
Neg. charged aa	1	1	1	1
Pos. charged aa	4	3	2	4
Net charge pH 7	4	2	2	3
% Neutral aa	32.14	33.33	33.33	33.33
% Basic aa	14.29	7.41	7.41	11.11
% Acid aa	3.57	3.70	3.70	3.70
% Hydrophobic aa	50.00	55.56	55.56	51.85

aa, amino acid

Half-life for these peptides was also predicted. For DRP-SP2 was 4.4 h in mammalian reticulocytes, more than 10 h in *E. coli* and more than 20 h in yeasts. DRP-SP3, DRP-SP4, and DRP-SP5 show a half-life of 1.9 h in mammalian reticulocytes, while their half-lives in *E. coli* and yeasts are the same as DRP-SP2. This allows, in a preliminarily way, to infer that Dermaseptins could be used in humans to fight microbial infections as their half-lives in humans are much lower than in bacteria.

The instability index estimates the stability of a protein in a test tube based on a statistical study on di-peptides in solution. In this investigation, a correlation between certain characteristics of a protein such as amino acid sequence, intrinsic properties, protease recognition mechanism, disulfide bridges, etc. with the capacity of the protein to suffer in vivo degradation was found. Moreover, a combination of some dipeptides was found to be an essential factor in stability of the protein [25]. DRP-SP2 has a value of 7.48; DRP-SP3 14.04; DRP-SP4 24.31; and DRP-SP5 28.67. Li et al. determined that proteins and peptides are predicted to be stable when an instability index value of less than 40 was obtained [26]. In this sense, these results showed that the peptides are predicted to be stable. This was expected since, in nature, it needs to be excreted through the skin of the frog, get in contact with the bacteria, and exert its function.

The flexibility in water for the four peptides was also predicted (Fig. 1). Protein flexibility is essential for molecular recognition. The secondary structure of peptides is crucial to bind to antibodies and produce the immune response [27]. Peptide flexibility should be high enough to produce

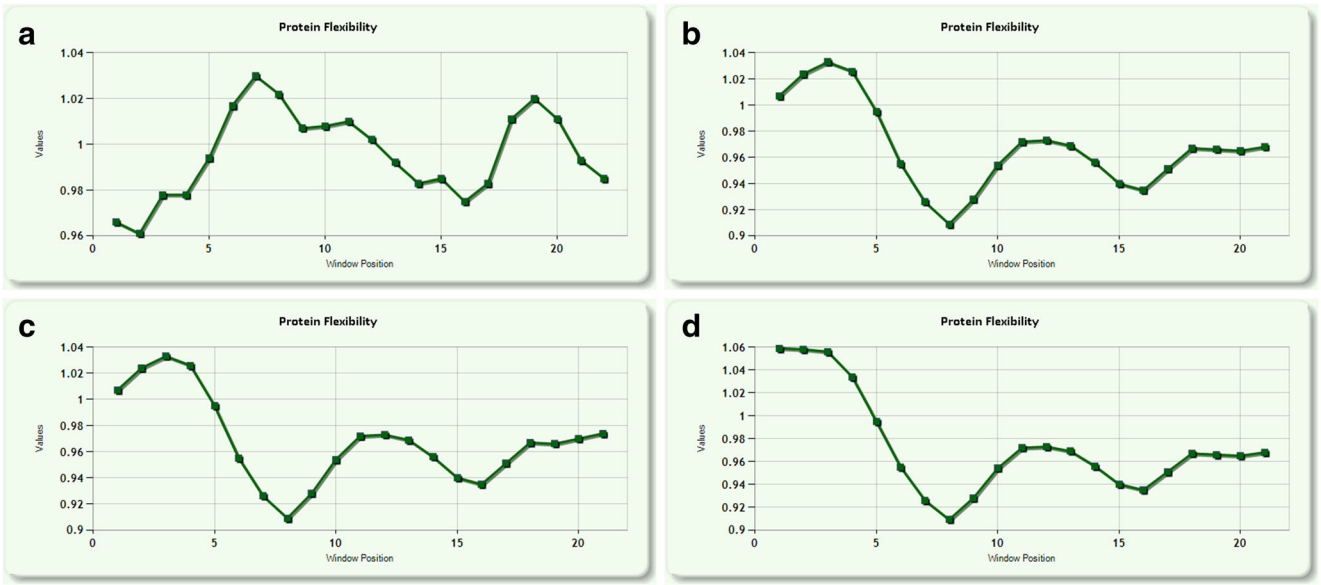

Fig. 1 Flexibility of the peptides according to their amino acid sequence [15]. **a** DRP-SP2, **b** DRP-SP3, **c** DRP-SP4, **d** DRP-SP5

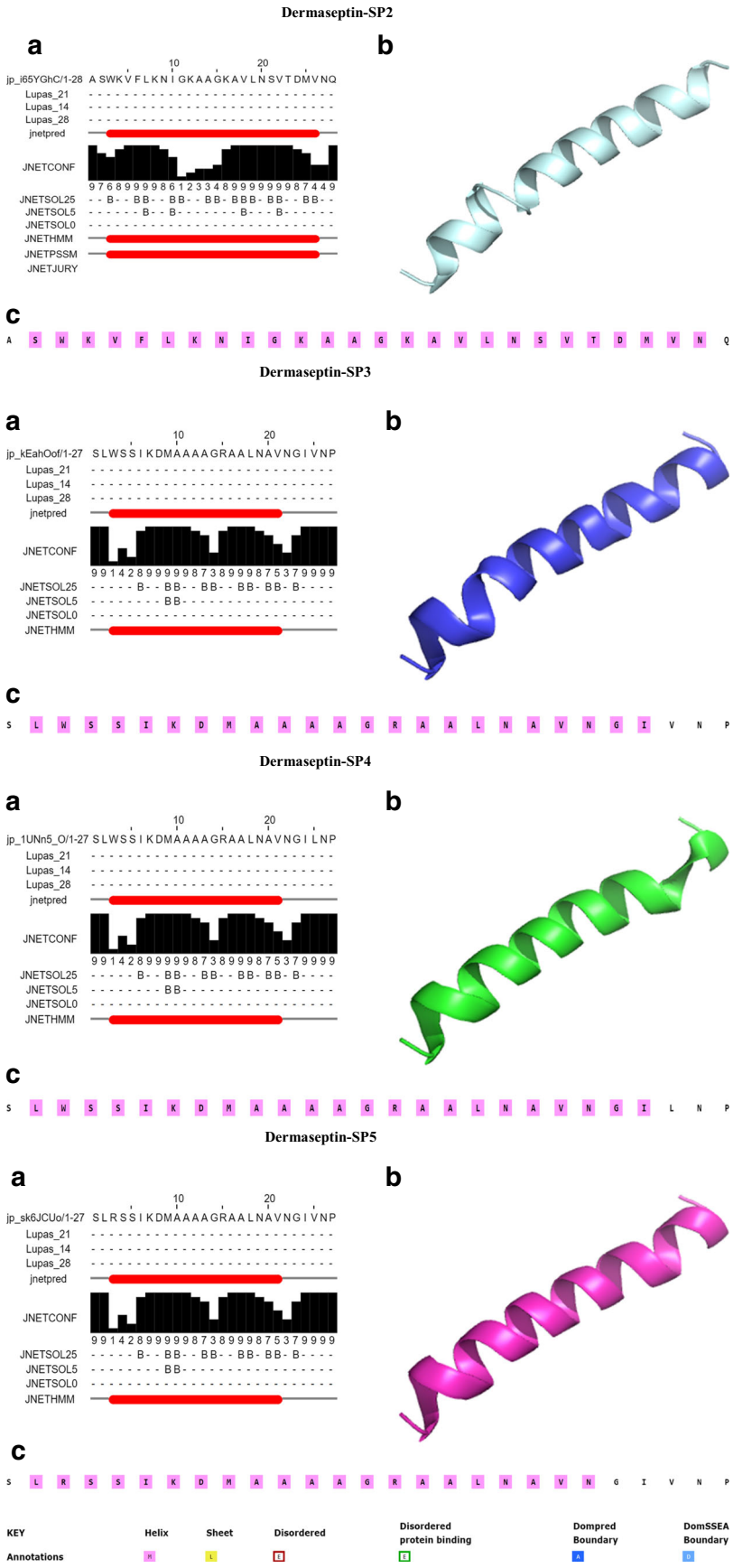

Fig. 2 Secondary structure of Dermaseptin-related peptides. **a** Prediction obtained from JPred. **b** Relaxation of the peptide with MM2 force field. **c** Prediction obtained from PSIpred

conformational changes to fit a specific binding pocket but not so flexible to lose its secondary structure affecting binding. Average flexibility value is 1. Values greater than 1 mean more flexible regions while values less than 1 show rigid regions [28]. Protein flexibility is determined basically by its amino acid sequence being glycine the most flexible and proline the least flexible amino acids. In this sense, large and branched residues reduce flexibility [29].

Flexibility prediction of the four Dermaseptin-related peptides showed that all the peptides have almost the same flexibility ranging from 0.96 to 1.03, but for DRP-SP2, the end zones are a little bit less flexible and the zones between the center and the end are slightly most flexible. For DRP-SP3, DRP-SP4, and DRP-SP5, the end zones and the area in the center of the peptides are the most flexible parts of the chain, while the two intermediate zones between them are the least flexible. This was expected, since in solution the zones with greater flexibility are the extremes. DRP-SP2 behaves differently as regards to the other peptides DRP-SP3, DRP-SP4, and DRP-SP5 due to its high difference in its amino acid sequence. Analyzing this more in depth, the presence of the big and rigid amino acid lysine in the first region makes DRP-SP2 be more rigid. Having two flexible glycine amino acids next to two lysines in the center makes the peptide flexibility values to be around 1 (0.98 to 1.02). Proline amino acid at the end of DRP-SP3, DRP-SP4, and DRP-SP5 makes the last region more rigid compare with DRP-SP2 that has a glutamine. Comparing DRP-SP3, DRP-SP4, and DRP-SP5, the change in the third amino acid in DRP-SP5 (Arginine) compare with the tryptophan found in DRP-SP3 and DRP-SP4 affects the flexibility of the peptide as arginine is more flexible than tryptophan. This makes the first end of DRP-SP5 slightly more

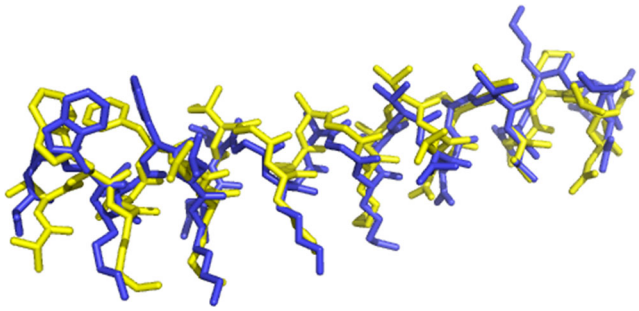

Fig. 4 Comparison between the relaxed structure (blue) using MM2 forcefield and the optimized one (yellow) using ONIOM HF/6-31G for DRP-SP2

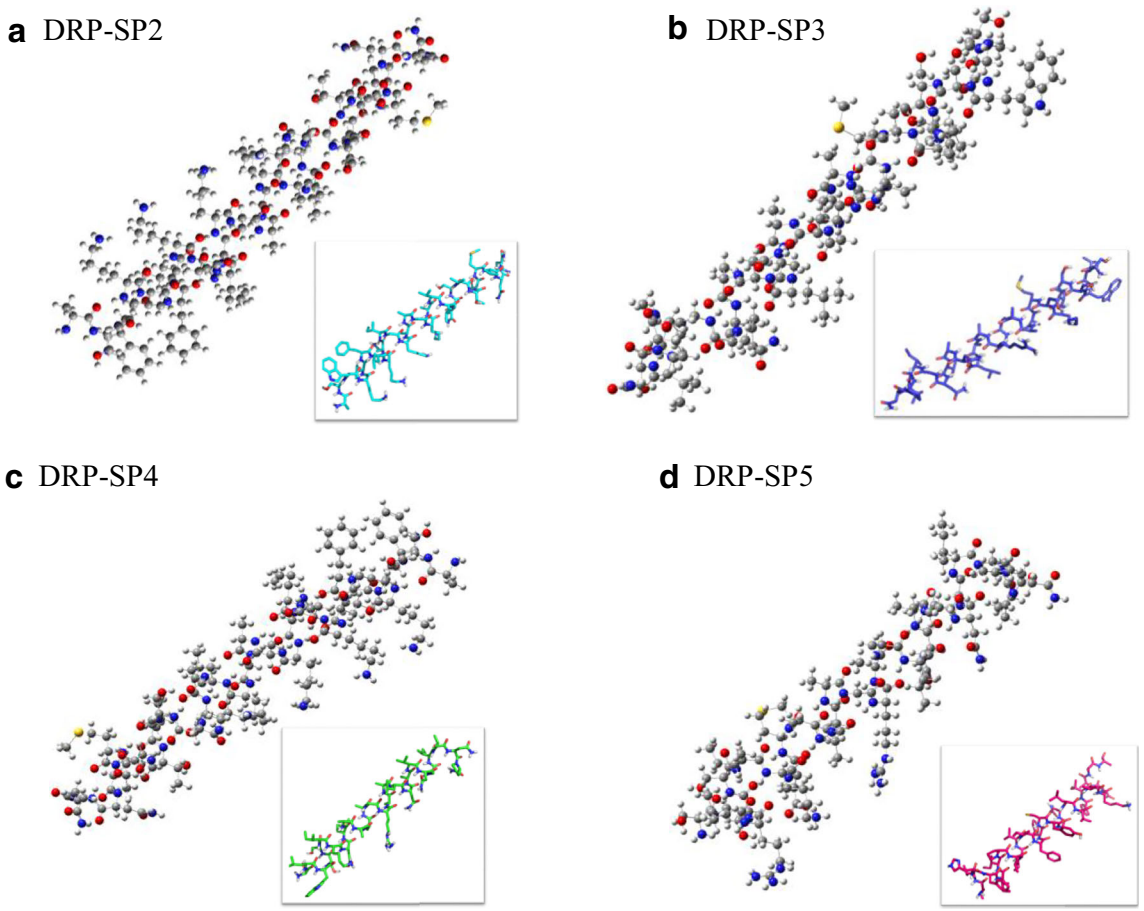

Fig. 3 Optimized structures obtained by ONIOM HF/6-31G quantum chemical method

Table 4 Similarity between different Dermaseptins and four Dermaseptin-related peptides in our study

Entry	Name	Organism	Length	% Identity			
				DRP-SP2	DRP-SP3	DRP-SP4	DRP-SP5
P84936	Dermaseptin-H6	*Phyllomedusa azurea*	29	38.1	61.9	66.7	57.1
P80280	Dermaseptin-4	*Phyllomedusa sauvagii*	27	44.4	40.0	40.0	36.0
P83637	Dermaseptin-01	*Phyllomedusa oreades*	29	38.1	61.9	66.7	57.1
P84922	Dermaseptin-2	*Phyllomedusa tarsius*	28	82.1	46.1	42.3	42.3
P84600	Dermaseptin-5	*Phyllomedusa hypochondrialis*	29	38.1	61.9	66.7	57.1

flexible than the other two. Changes of the third last amino acid leucine in DRP-SP4 for valine in DRP-SP3 and DRP-SP5 do not change the flexibility in the last end region because the difference between these amino acids is just one carbon having both amino acids contributing almost the same flexibility.

A more detailed study using molecular dynamic techniques is necessary to validate this prediction.

For their secondary structure, all three techniques used (JPred, PSIpred, and the relaxed peptides obtained from ChemDraw) predicted that these molecules tend to form an alpha helix (Fig. 2). From now on, DRP-SP2 is presented in cyan, DRP-SP3 in blue, DRP-SP4 in green, and DRP-SP5 in pink. The advantages of JPred and PSIpred lie on their accuracy being more than 75% [16, 17]. On the other hand, the optimized structure from ChemDraw has a disadvantage where the input structure is already in its alpha helical structure. This method is useful to relax the peptide's structure after having some knowledge about the secondary structure, but is not recommended for structures with unknown secondary structure. In this research, ChemDraw was used because an alpha helical structure for Dermaseptins was found in the literature [30]. In addition, the geometry optimized by quantum mechanical calculations confirms the structure described by the programs used.

The three methods used to predict secondary structure show that Dermaseptin-related peptides are going to form an alpha helix. This result is in agreement with experimental data found in the literature where peptides of the Dermaseptin family have an α-helical secondary structure [30]. However, it is necessary to carry out structural elucidation studies of the peptides to confirm the results obtained by molecular modeling. Figure 2 shows a great correlation in the three methods for DRP-SP3, DRP-SP4, and DRP-SP5. DRP-SP2 shows small differences between the methods, where JPred predicts an alpha helix throughout its structure, but for the first two and the last two amino acids. PSIpred, on the other hand, shows an alpha helical structure throughout the peptide, except for the first and last amino acid. Finally, the relaxed structure shows an alpha-helical peptide in the whole sequence, except for the first amino acid, the last two, and amino acid 9 and 10 (Asparagine, Isoleucine).

A more accurate and computational demanding method was used to optimize the peptides at a quantum level (Fig. 3).

The results showed the alpha helical backbone structure is maintained when comparing the relaxed structure with the

Table 5 Score values between Dermaseptin-related peptides and different enzymes compared with known inhibitors

Organism	Enzyme	Known inhibitor	Score (kcal/mol)				
			Known inhibitor	DRP-SP2	DRP-SP3	DRP-SP4	DRP-SP5
S. aureus	G acyl-penicillin binding protein 2A (PDB:1MWT)	Ceftobiprole	− 9.5	− 5.1	− 5.3	− 5.3	− 4.8
	Hydrolase AmiA (PDB:4KNL)	Muramyl tetrapeptide	− 7.1	− 5.0	− 5.8	− 6.1	− 5.5
E. coli	DNA gyrase B (PDB:4PRV)	ADP	− 10.4	− 4.2	− 4.1	− 4.9	− 4.8
	Transglycosylase penicillin binding protein 1B (PDB:3VMA)	Moenomycin	− 7.3	− 5.1	− 5.5	− 5.5	− 5.2
C. albicans	Exo-B-(1,3)-glucanase (PDB:1EQC)	Castanospermine	− 7.0	− 5.3	− 5.9	− 3.8	− 4.3
	Secreted aspartic proteinase (PDB:1EAG)	A70*	− 7.7	− 6.1	− 6.4	− 6.4	− 6.5

*A70: N-ethyl-N-[(4-methylpiperazin-1-yl)carbonyl]-D-phenylalanyl-N-[(1S,2S,4R)-4-(butylcarbamoyl)-1-(cyclohexylmethyl)-2-hydroxy-5-methylhexyl]-L-norleucinamide

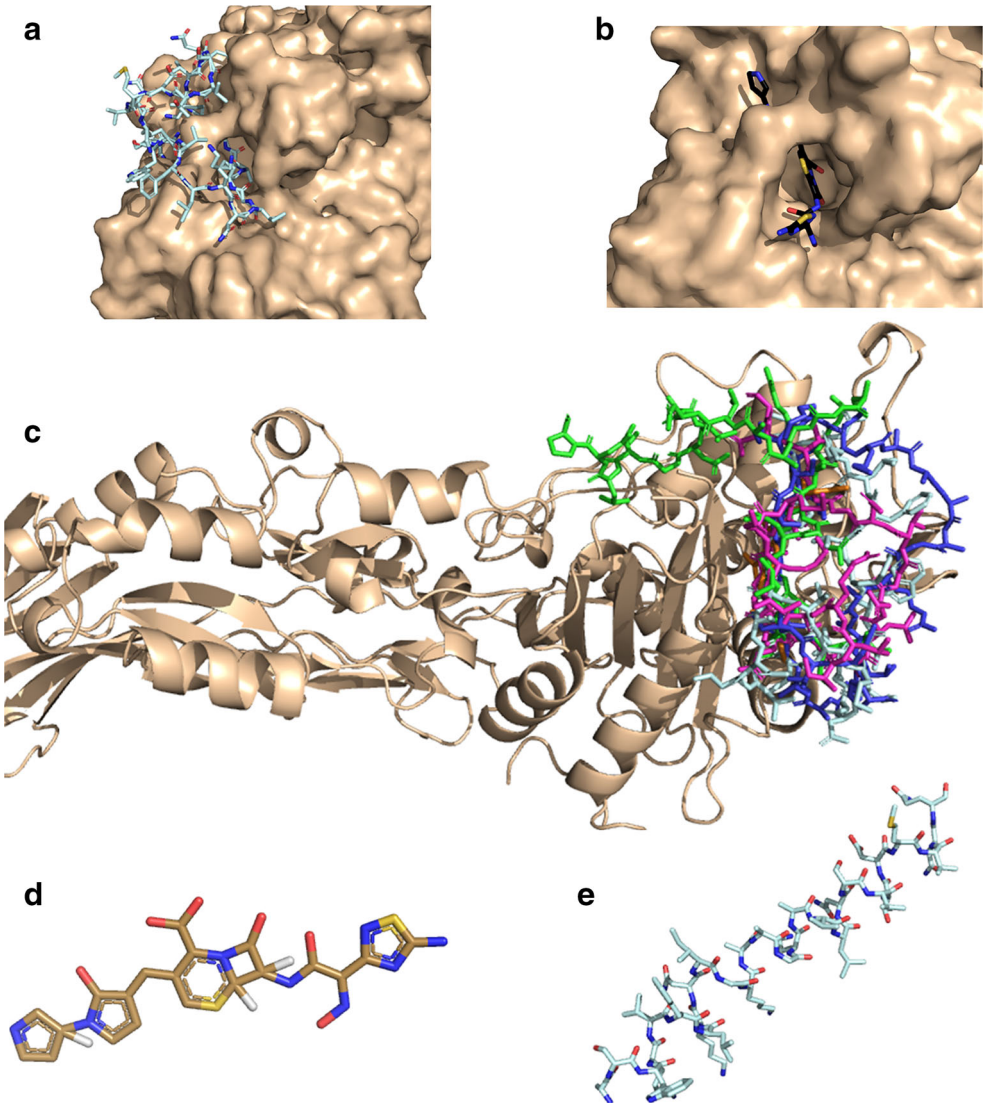

Fig. 5 G acyl-penicillin binding protein 2A docking. **a** DRP-SP2 docking. **b** Ceftobiprole docking. **c** Interaction with the four Dermaseptins and Ceftobiprole. **d** Ceftobiprole. **e** DRP-SP2

optimized one for all the four peptides (Fig. 4). Small differences can be noticed in the peptide sidechains where spatial conformation changes due to hydrogen interactions taken into account in quantum chemical calculations. The behavior found was the same for all the four peptides; therefore, only DRP-SP2 structure was depicted in Fig. 4.

Dermaseptin-related peptides were compared with other peptides in different species to determine their similarity. Using Uniprot database, hundreds of Dermaseptin-related peptides discovered in other frog species were compared with the ones obtained from *Agalychnis spurrelli*. The similarity of the Dermaseptin-related peptides ranges from 36.0% between DRP-SP5 and Dermaseptin-4 from *Phyllomedusa sauvagii* up to 82.1% between DRP-SP2 with Dermaseptin-2 from *Phyllomedusa tarsius*. The results are shown in Table 4.

The identity study of the four peptides demonstrated that the isolated structures belong to the family of the Dermaseptins having similar length and secondary structure. The greatest similarity was found between DRP-SP2 and Dermaseptin-2 extracted from the tarsal frog (*Phyllomedusa tarsius*) which also can be found in Ecuador. It would be interesting to investigate at a genetic level what the relationship is between these two species and how this similarity can determine the structure of their microbial defense peptides.

Next, the mechanism of action of these peptides was studied. Two options were taken into account. The first one is through inhibition of one or more enzymes of a vital biological pathway of the bacteria. Second one is cell membrane lysis caused by charged interaction of the peptides.

Two known enzymes of each studied organism were chosen. Those enzymes are known as biological targets for several antimicrobial molecules. Docking results show that, in every case, these peptides have less score (called affinity in Autodock VINA) compared with their known inhibitor (Table 5). In this investigation, we will talk about scores rather than affinity in the docking studies because using docking is

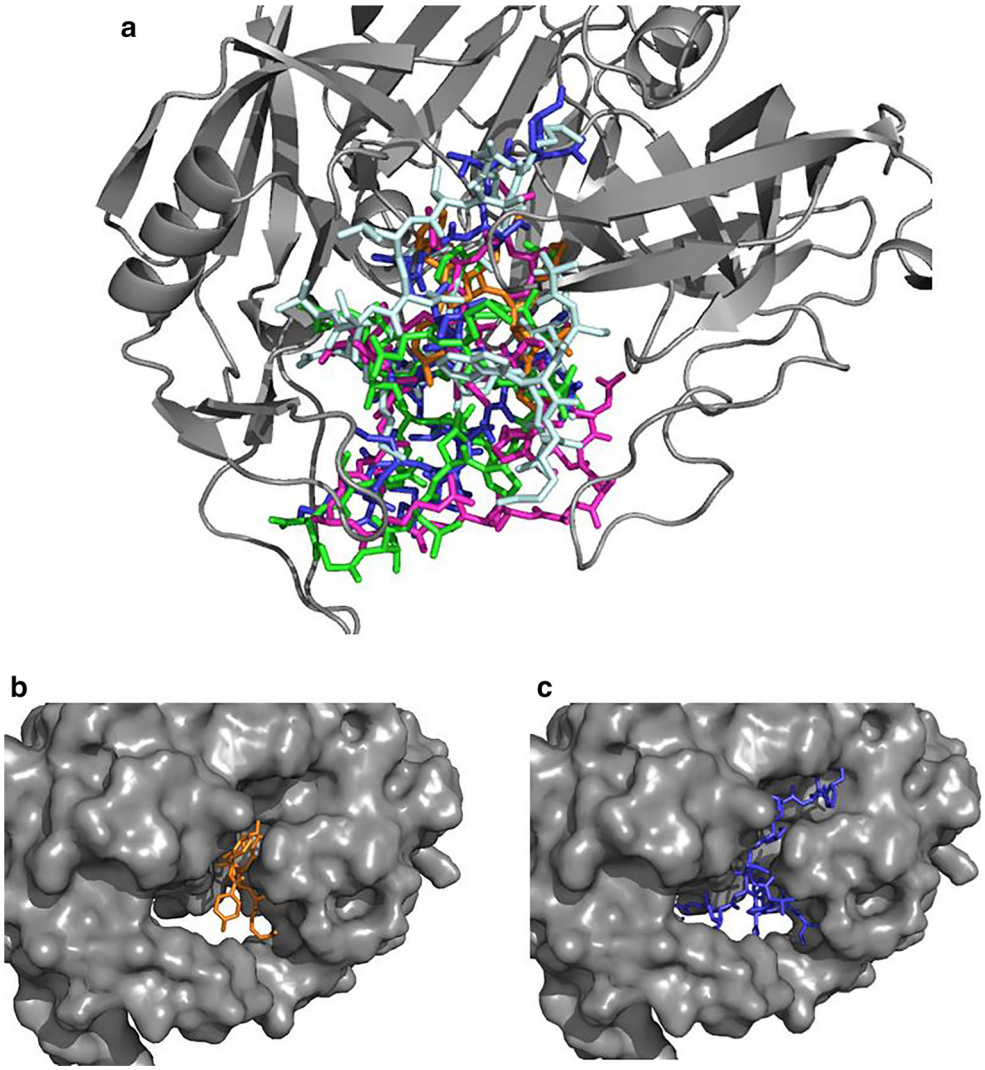

Fig. 6 Secreted aspartic protease docking. **a** Docking with the four Dermaseptins and A70. **b** A70 docking. **c** DRP-SP3 docking

not possible to produce an accurate binding affinity value. To produce affinity values, the effect of multiple protonation states plus the energy of the unbound enzyme ligand complex must be obtained. Docking scores will therefore classify active from inactive binders making experimental studies less expensive [31, 32]. Therefore, we can infer that the mechanism of action of Dermaseptins is not given by inhibition of any of the proposed enzymatic pathways as they appear as inactive molecules towards the enzymes.

Table 6 Docking score values of the interaction between Dermaseptin-related peptides with mammalian and bacterial cell membrane

Peptide	Score (kcal/mol)	
	Mammalian cell membrane (PDB:2MLR)	Bacterial cell membrane (PDB:2W6D)
DRP-SP2	− 3.2	− 4.0
DRP-SP3	− 4.7	− 5.1
DRP-SP4	− 3.7	− 3.2
DRP-SP5	− 3.6	− 3.2

The enzymatic interaction was analyzed more in depth. In the case of penicillin G-acyl binding protein 2A, one of its known inhibitors is ceftobiprole. This drug belongs to the family of cephalosporin [33]. In enzyme-drug interactions, charge and size of the inhibitor is key to produce inhibition. For this antibiotic, its charge at physiological pH is − 1. This charge is opposite when comparing with any of the studied Dermaseptins that have a positive charge at pH 7. In the case that Dermaseptins partially manage to enter the enzyme pocket charge difference will prevent the interaction (Fig. 5).

For AmiA Hydrolase, both the inhibitor (Muramyl tetrapeptide) and Dermaseptins are basic peptides with a difference in their length (4 vs. 27+ amino acids). The distance between the basic amino acids in muramyl tetrapeptide allows this peptide to achieve a greater affinity in the active site of the AmiA hydrolase compared with Dermaseptins.

DNA Gyrase B is an enzyme that reduces the strain of DNA strands during their replication. The inhibitors will attack the

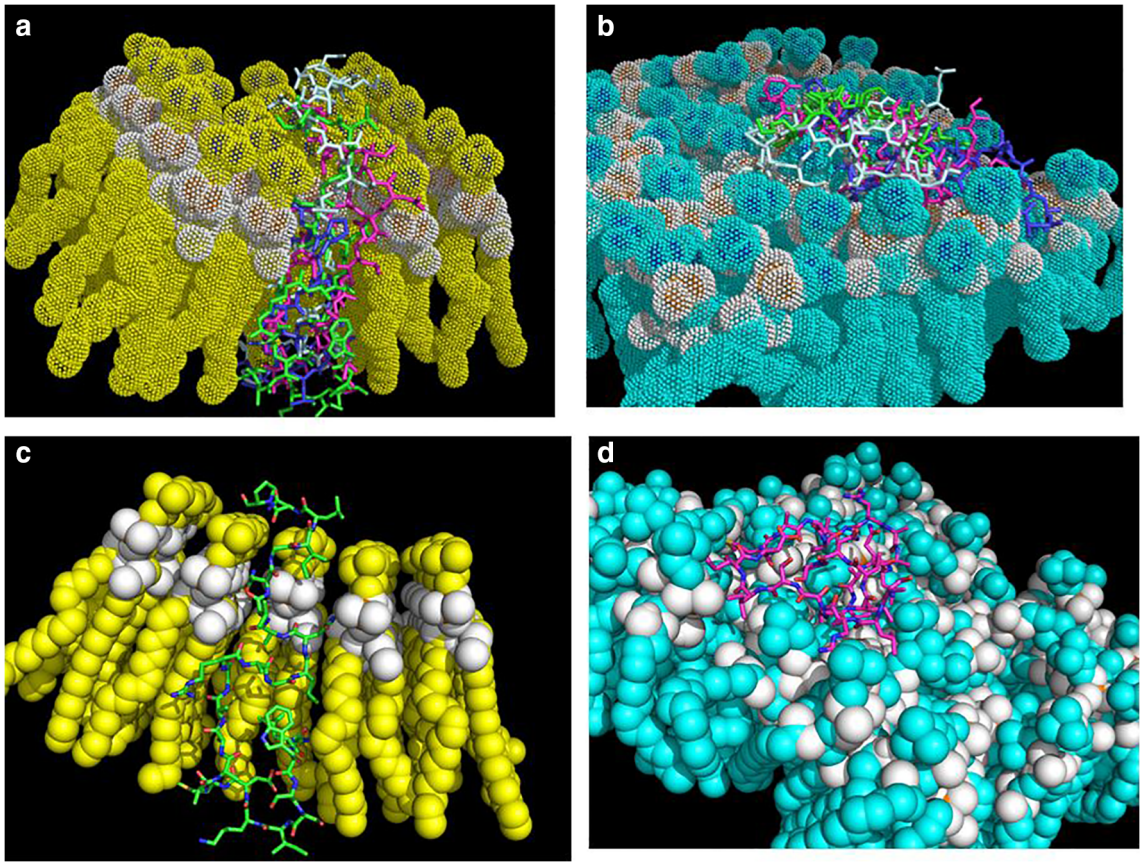

Fig. 7 Interaction of four Dermaseptins with **a** mammalian cell membrane, **b** bacterial cell membrane, **c** DRP-SP4 interaction with bacterial membrane, and **d** DRP-SP5 interaction with mammalian cell membrane

binding site of this enzyme with ADP. This nucleotide, due to the phosphate groups, is a negatively charged molecule, which is opposite to the molecules studied, explaining their low score.

The difference in affinities between the inhibitor and Dermaseptins extracted from *Agalychnis spurrelli* in transglycosylase penicilin binding protein 1B, is given by charge distribution, in the same way as penicillin G-acyl binding protein 2A.

The secreted aspartic protease has as inhibitor molecule A70. Proteases are enzymes that break peptide bond. This explains why the difference in the score between A70 with Dermaseptins is only 1.5 kcal/mol, being the lowest of all the

Table 7 Docking score of the interaction between Dermaseptin-related peptides with molecules present in the cell membrane

Cell membrane molecules/peptides	Score (kcal/mol)			
	DRP-SP2	DRP-SP3	DRP-SP4	DRP-SP5
Teicoic acid	− 6.3	− 6.2	− 5.7	− 5.6
beta 1–3 glucane	− 4.0	− 3.8	− 3.4	− 3.5
Cardiolipin	+ 8.5	− 1.7	− 1.9	− 1.1
Phosphatidylethanolamine	− 7.0	− 6.7	− 6.6	− 6.7
Glycophospholipids phosphomanolipid	− 5.8	− 5.5	− 5.6	− 5.7
Glicofosfolipido GPLs	− 7.9	− 7.2	− 7.2	− 7.3
Lysyl-phosphatidylglycerol	− 6.4	− 5.9	− 6.1	− 6.0
Miristic acid	− 4.6	− 4.2	− 4.6	− 4.4
Oleic acid	− 5.4	− 5.4	− 5.8	− 5.1
Palmitic acid	− 5.2	− 4.6	− 5.0	− 4.8
Palmitoleic acid	− 5.1	− 5.0	− 5.6	− 4.8
Phosphatidylglycerol	− 7.1	− 6.5	− 6.5	− 6.4

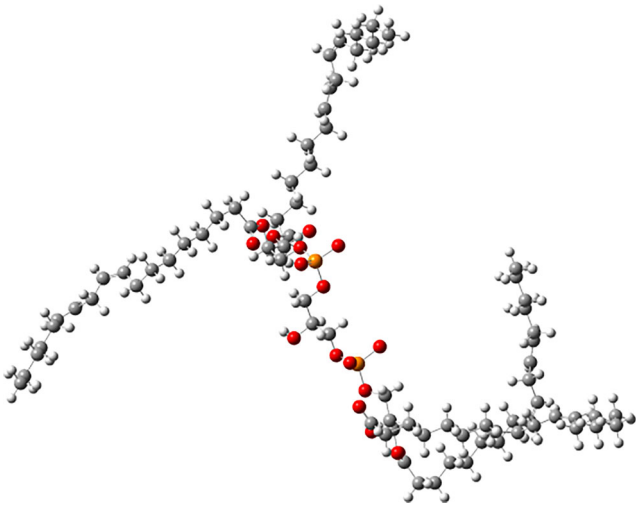

Fig. 8 Cardiolipin molecular structure

enzymes studied. Peptides could not be used as inhibitors since these structures are the enzyme's natural substrate fitting exactly in the enzymatic pocket (Fig. 6). Dermaseptins are going to be hydrolyzed in the presence of this enzyme.

To study the mechanism of action through cell lysis, molecular docking was performed between Dermaseptins, mammalian, and bacterial cell membranes. The gridbox or docking space chosen was all the membrane to determine in which part of the membrane the peptides will be more likely to interact. The results show a higher score of the peptides towards the bacterial cell membrane for DRP-SP2 and DRP-SP3. In DRP-SP4 and DRP-SP5 interaction, there is a greater score towards the mammalian cell membrane (Table 6). Computational calculations always have a margin of error. Because of this, as the differences in the scores are within 1 kcal/mol, it can be said that score values are the same for both membranes in all cases.

Analyzing the interaction coordinates (Fig. 7), it is clearly seen that the peptides' top-scoring poses occur inside the membrane channel by penetrating the bacterial cell membrane; while all ten poses for each studied peptide interacting with the mammalian cell membrane occurs on the surface. This may suggest that Dermaseptins will prefer to stay in the mammalian membrane surface, while they may penetrate the bacterial membrane. Although this is just a preliminary outcome, these results agree with the antimicrobial mechanism of action proposed for this type of Dermaseptin peptides and their low toxicity found towards humans cells [1, 3, 5, 6, 8, 34, 35]. The opposite will happen in the bacteria, wherein penetrating the membrane will break it, causing cell lysis and killing of the microorganism.

Further analysis of Dermaseptins docking against molecules present in the cell membrane of the three studied microorganisms was performed. Twelve molecules were chosen showing scores from − 1.1 to − 7.9 kcal/mol (Table 7).

Scores remain similar in all cases with the exception of cardiolipin, which presents a different score between DRP-SP2 and DRP-SP3, DRP-SP4 and DRP-SP5. As Dermaseptins are basic peptides and the studied molecules present in the cell membrane are negatively charged either by an acid group (COO^-) or a phosphate group (PO_4^{-3}), there is an acid-base interaction between them. The score will depend on the size and how exposed is this negative group. In the case of the cardiolipin (Fig. 8), the score is the lowest because the molecule is too bulky making it hard to interact with the basic peptides. DRP-SP2 reaches a positive value because there is no interaction at all. This was expected because this peptide has 4 lysine amino acids which are very long making it harder to get through cardiolipin long carbon chains and produce an interaction.

Conclusions

Dermaseptins, extracted from *Agalychnis spurrelli*, are short-chain cationic peptides that have different effects on Gram-positive, Gram-negative, and fungal bacteria. It was found that DRP-SP2 has activity against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. DRP-SP3 and DRP-SP4 possess antimicrobial activity against *E. coli* and *S. aureus* while the DRP-SP5 against *E. coli* and *C. albicans*. Physicochemical property prediction showed they are alpha-helical peptides with isoelectric points greater than 9.7 and a charge that ranges from + 2 to + 4 at physiological pH. Docking studies suggested that the possible mechanism of action of this peptide is not given by the inhibition of vital enzymatic pathways for the microorganism. Instead, by cellular lysis caused by the interaction of these basic peptides with the negative charged bacterial cell membrane. Comparison of the studied peptides against Dermaseptins of other species showed that these amino acid sequences are not found in any other species reported to date. Thus, these are novel molecules that could be studied more thoroughly as a starting point for next-generation antimicrobial drugs.

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