Molecular modeling of the vasopressin V1b receptor and its interaction with a selective antagonist

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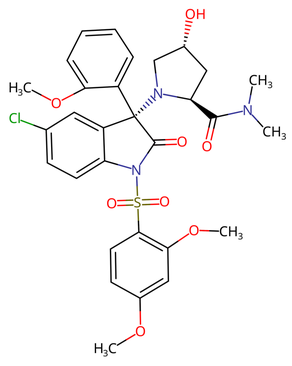
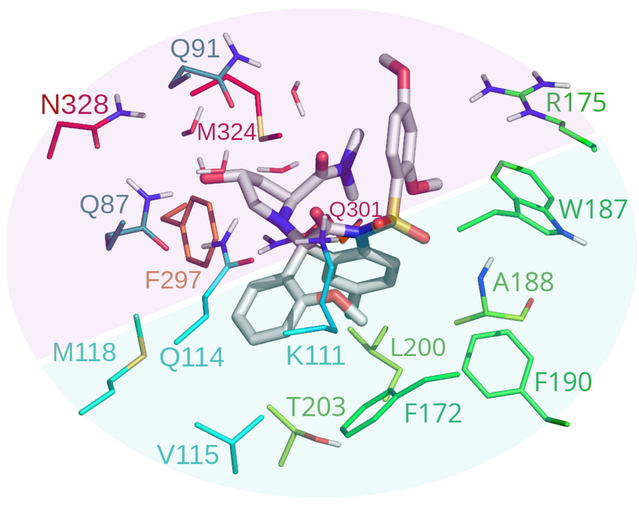


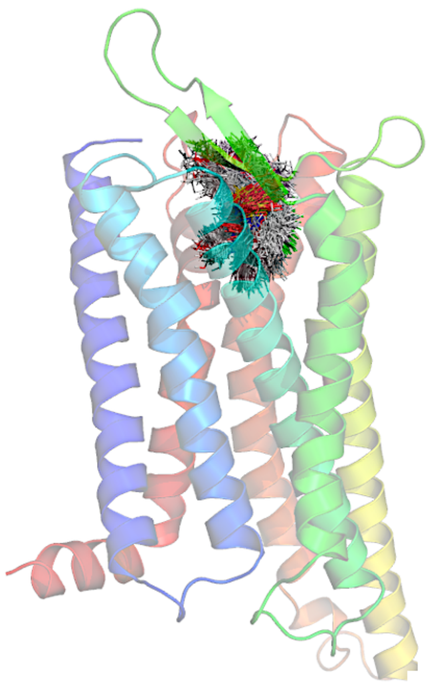
INTRODUCTION

The human V1b receptor (V1bR) belongs to the G protein-coupled receptors. The activation of V1bR induces the secretion of adrenocorticotropin hormone (ACTH) from the anterior pituitary cells, which in turn, stimulates the production of cortisol via the adrenal cortex. The chronic dysfunction of the hypothalamic-pituitary-adrenal (HPA) axis is correlated with several psychiatric disorders. Therefore, the inhibition of the V1b receptor and normalizing the HPA axis activity is a promising approach to the treatment of stress-related disorders such as anxiety and depression. Nelivaptan is a selective V1bR antagonist which may be used for treating depressive and anxiety disorders. It is also an excellent model molecule to study V1bR interaction with antagonists. In recent years the experimental structures of vasopressin V2 and oxytocin receptors were solved, providing excellent templates for homology modeling of V1bR. The analysis of the receptor-ligand interaction allows to get insights into the molecular mechanism of antagonist binding to V1bR.

METHODS

RECEPTOR: V1br (UniPROT no. P47901) was built via a SWISS-MODEL server. A 1 μs long molecular dynamics (MD) simulation was performed in fully hydrated membrane built out of POPC and cholesterol at a ratio of 4:1. **LIGAND**: The three-dimensional model of nelivaptan was built using the coordinates from the PubChem database (CID: 9895468). The parameters of nelivaptan were obtained using Antechamber with the general AMBER force field (GAFF). **DOCKING AND MD:** Nelivaptan was docked into V1bR using AutoDock 4.2.6. The lowest-energy complex was selected for MD simulation. The complex was embedded into the membrane of a composition identical to that used for the unliganded V1bR. MD was carried out for 1.5 μs using the computers of the Centre of Informatics Tricity Academic Supercomputer & Network. **ANALYSIS:** The trajectory was processed using the cpptraj module from the AMBER package.





RESULTS

The receptor-nelivaptan complex remained stable throughout the entire MD simulation. The receptor-ligand linear interaction energy (LIE) was negative throughout the entire simulation, which indicates a strong attraction between V1bR and nelivaptan. The average total LIE is -96.8 kcal/mol. The most important interactions responsible for anchoring nelivaptan inside the V1bR binding cavity are the hydrogen bonds formed by conserved polar residues with long side chains (Table). These hydrogen bonds are the main reason why nelivaptan does not leave the spacious receptor pocket wide open into the extracellular space. The stability of the V1bR-nelivaptan complex is enhanced by the conserved aromatic/hydrophobic residues. Among them, W187$^{45.51}$ and A188$^{45.52}$ are part of the DCWA motif observed within the vasopressin/oxytocin receptor family. Several residues interacting with nelivaptan are nonconserved. Two residues, L200$^{5.39}$ and T203$^{5.42}$, which are located one turn of the helix from each other and interact with the chlorine atom of oxindol moiety may be responsible for the selective binding of antagonists in V1bR.

V1br residue	Nelivaptan group	Contact type	LIE [kcal/mol]
Q87$^{2.57}$	pyrrolidine	hydrogen bond	-2.4
Q91$^{2.61}$	pyrrolidine, oxindol	hydrogen bond	-0.7
R99$^{23.49}$	dimethoxyphenyl	hydrogen bond	-0.3
K111$^{3.29}$	oxindol, sufonyl	hydrogen bond	-46.3
Q114$^{3.32}$	methoxyphenyl, oxindol	hydrogen bond	-3.6
V115$^{3.33}$	methoxyphenyl	aromatic-hydrophobic	-2.0
M118$^{3.36}$	methoxyphenyl	aromatic-hydrophobic	-1.6
F172$^{4.64}$	methoxyphenyl	aromatic	-2.3
R175^{ECL2}	dimethoxyphenyl	hydrogen bond	-0.8
W187$^{45.51}$	dimethoxyphenyl	aromatic	-8.3
A188$^{45.52}$	sulfonyl, methoxyphenyl	hydrogen bond, hydrophobic	-2.7
F190^{ECL2}	oxindol	aromatic	-2.0
Y199$^{5.38}$	oxindol	aromatic	-2.3
L200$^{5.39}$	oxindol	VdW/ aromatic-hydrophobic	-0.9
T203$^{5.42}$	oxindol, methoxyphenyl	VdW/ aromatic-hydrophobic	-1.1
F297$^{6.51}$	pyrrolidine	aromatic-hydrophobic	-2.8
Q301$^{6.55}$	N-methyl	Van der Waals/hydrophobic	-4.3
M324$^{7.39}$	N-methyl, pyrrolidine	hydrophobic	-2.1
N328$^{7.43}$	pyrrolidine	hydrogen bond	-2.6

Structural characterization of the arginine-rich gemini lipopeptides containing p-xylene bridge

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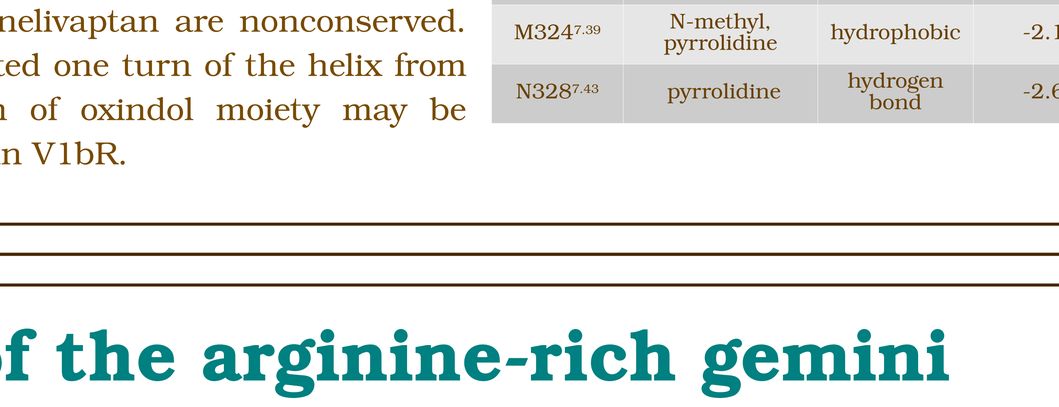
INTRODUCTION

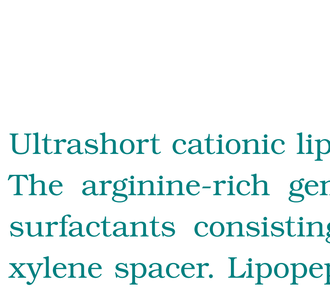
Ultrashort cationic lipopeptides are potent antimicrobial and antifungal agents. The arginine-rich gemini lipopeptides containing p-xylene bridge are gemini surfactants consisting of two surfactant molecules bonded together by a p-xylene spacer. Lipopeptide antibiotics, such as daptomycin and polymyxins are often used as drugs of last-resort in the treatment of systemic infections caused by susceptible strains of multidrug-resistant organisms such as *Pseudomonas aeruginosa* or *Staphylococcus aureus*. The mechanism of lipopeptides' action is still not fully understood. In general, they interact with the bacterial or fungal cell membrane and disrupt the membrane integrity, initiating a series of events that eventually leads to the cell's death. Understanding this mechanism at the molecular level is crucial for designing novel lipopeptide agents with improved antimicrobial activity. In this work, molecular dynamics (MD) simulations were performed to investigate the mechanism of interaction of the lipopeptides with bacterial membrane and to test their self-assembly tendency.



METHODS

The lipopeptide structures were constructed *de novo*. The non-standard fragments (Figure above) were parameterized using GAMESS and RESP programs. The raw structures were energy-minimized and 200 ns of molecular dynamics in implicit water was performed. A model of a Gram-positive bacterial membrane was built using the POPG and POPE lipids at a ratio of 3:1. The lipopeptides were placed randomly above the membrane's surface, after which the system was hydrated with TIP3P water and neutralized with Na+ counterions. The molecular dynamics simulation was carried out for 25 ns. The second simulation was performed in a TIP3P water box to test the self-assembly tendency of the lipopeptides in water. The box contained one hundred lipopeptides (with RR:RS:SS ratio of 1:2:1) placed inside the box using Packmol. The MD simulations were performed using the computers of the Centre of Informatics Tricity Academic Supercomputer & Network.

To determine the antimicrobial activity of the investigated lipopeptides against *S. aureus*, *P. aeruginosa* and *C. albicans*, minimal inhibitory concentrations (MIC) testing was performed. To assess the toxic side effects of the compounds against human cells, standard hemolysis assays were performed.



RESULTS

The arginine-rich gemini lipopeptides containing p-xylene bridge exhibited antimicrobial activity against all tested bacteria (Table) and the highest activity was observed against *S. aureus* and *C. albicans*. Gram-negative *P. aeruginosa* is more resistant to lipopeptides than Gram-positive bacteria and fungi which is consistent with previous studies on lipopeptides.

The molecular dynamics simulations in water and near the bacterial membrane reveal quick lipopeptides self-assembly into micelles consisting of two to four lipopepides. In addition, during the first nanoseconds of the simulation with membrane, the lipopeptides flowed to the bilayer surface and interact with the polar lipid heads. In the next steps, the arginine side chains started to penetrate into the lipids.

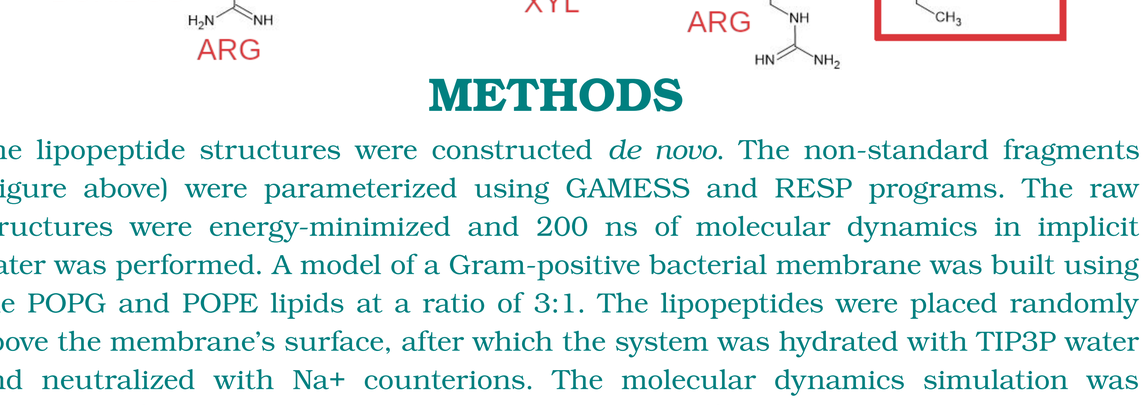
The MD simulation has now been extended to further investigate how the micelles of arginine-rich gemini lipopeptides affect the structure of bacterial membrane and how it is correlated with their antimicrobial activity.

ANTIMICROBIAL ACTIVITY

	S. aureus	*P. aeruginosa*	*C. albicans*
MIC [μg/ml]	4	64	16

CYTOTOXICITY

HC50 [μg/ml]
85.09 ± 1.33



Lipopeptides before (top) and after (bottom) the simulation in water and bacterial membrane.