SGTx1, a Kv Channel Gating-Modifier Toxin, Binds to the Interfacial Region of Lipid Bilayers

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ABSTRACT SGTx1 is a gating-modifier toxin that has been shown to inhibit the voltage-gated potassium channel Kv2.1. SGTx1 is thought to bind to the S3b-S4a region of the voltage-sensor, and is believed to alter the energetics of gating. Gating-modifier toxins such as SGTx1 are of interest as they can be used to probe the structure and dynamics of their target channels. Although there are experimental data for SGTx1, its interaction with lipid bilayer membranes remains to be characterized. We performed atomistic and coarse-grained molecular dynamics simulations to study the interaction of SGTx1 with a POPC and a 3:1 POPE/POPG lipid bilayer membrane. We reveal the preferential partitioning of SGTx1 into the water/membrane interface of the bilayer. We also show that electrostatic interactions between the charged residues of SGTx1 and the lipid headgroups play an important role in stabilizing SGTx1 in a bilayer environment.

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SGTx1 is 34-residue peptide toxin from the tarantula venom (1), which is homologous to HATx1, the first gating-modifier toxin to be identified (2,3). It is a stable, globular structure composed of an antiparallel β-sheet stabilized by disulphide bridges. SGTx1 inhibits the voltage-gated (Kv) potassium channel Kv2.1 by binding to the S3b-S4a region of the voltage sensor (VS) domain, altering the energetics of voltage activation (1). The active surface of SGTx1 is thought to contain both hydrophobic and charged residues. SGTx1 is amphipathic: one half of its surface consists predominantly of hydrophobic residues, whereas its other half consists predominantly of polar residues. This appears to be conserved across different gating-modifier toxins (4,5), suggesting a common mode of access and binding to the VS.

The mechanism of voltage-dependent gating of Kv channels remains controversial (6). The nature of the conformational change that the VS domain undergoes during gating and how this movement is coupled to the pore domain is unclear. Several models of gating have been proposed, which differ in the degree of movement of the gating charges located on the voltage-sensing S4 helix. Gating-modifier toxins such as SGTx1 provide an approach to probing the structure and dynamics of the VS (6,7). Because of the presence of both hydrophobic and basic residues on the surface of the toxin, gating-modifier toxins such as SGTx1 and VSTx1 have been proposed to gain access to the binding site on the VS domain by partitioning into the lipid bilayer membrane (6,8–10), close to the headgroups of anionic lipids. We recently used atomistic molecular dynamics (MD) simulations to investigate the interaction of VSTx1, a gating-modifier toxin that inhibits the archael channel KvAP, with lipid bilayers (11). VSTx1 and SGTx1 appear to share a conserved structure; therefore we anticipate the two toxins may interact with lipid bilayers in a similar fashion, namely via binding at the bilayer/water interface, enabling the toxin molecule to interact with both the hydrophobic tails and the polar headgroups of the lipid molecules. Here, we focus on SGTx1 using a combination of atomistic and coarse-grained (CG) simulations to provide a detailed view of its interactions with zwitterionic and anionic lipid bilayers.

We performed MD simulations to study the interaction of SGTx1 with a POPC (SGTX-PC) and a 3:1 POPE/POPG (SGTX-PEPG) bilayer membrane. MD simulations were performed using GROMACS (www.gromacs.org). SGTx1 was kept in the default protonation state for pH 7 in all simulations. In the atomistic simulations (each of 10 ns duration), we harmonically restrained SGTx1 at six different initial depths in the bilayer (Fig. 1). The six depths correspond to: i), two locations with the toxin completely buried within the hydrophobic core of the bilayer ($z = 0$ and 3 Å; distances measured from the midpoint of the bilayer; the z-axis corresponds to the bilayer normal); ii), two locations with the toxin spanning the hydrophobic core and the headgroup/water interface ($z = 9.5$ and 16.5 Å); and iii), two locations with the toxin between the headgroup region and the adjacent aqueous phase ($z = 23.5$ and 30.5 Å). At all depths, SGTx1 was initially orientated such that its hydrophobic half was exposed to the hydrophobic core of the membrane. CG approaches offer the opportunity to explore timescales inaccessible with traditional atomistic simulations (12). We performed two sets of three CG MD (13) simulations (each of 0.2 μs duration) to probe the dynamics of SGTx1 interacting with a POPC (SGTX-PC-CG) and with a 3:1 POPE/POPG (SGTX-PEPG-CG) bilayer. For these, SGTx1 was

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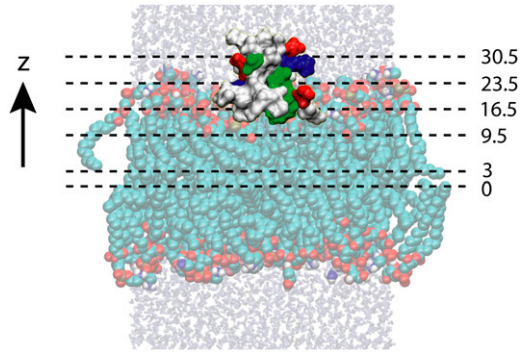

FIGURE 1 Starting depths of the toxin for the atomistic simulations (SGTx1 in POPC shown). The *z* axis of the simulation box corresponds to the bilayer normal. Distances (in angstroms) are relative to the bilayer center of mass. SGTx1 is shown at a starting depth of 23.5 Å. The hydrophobic, basic, acidic, and polar residues of SGTx1 are shown in green, blue, red, and white, respectively. POPC carbon, oxygen, nitrogen, and phosphorus atoms are shown in cyan, red, blue, and gold, respectively. The water molecules are shown in light blue.

initially positioned in the aqueous environment close to the surface of the bilayer.

In both the SGTX-PC and SGTX-PEPG atomistic simulations, there is a degree of displacement of the toxin along the bilayer normal despite the harmonic restraint applied to the center of mass of the toxin. This indicates a tendency for the toxin to move toward a more favorable location of interaction. We examined the average displacement of the center of mass of the toxin from the different toxin starting depths over the simulation period. The overall directionality of movement (Fig. 2) suggests a preferred toxin depth of ~23.5 Å for both SGTX-PEPG and SGTX-PC (data not shown). The overall proposed location suggests that SGTx1 prefers to be located close to the membrane/water interface (headgroup/water interface) of the bilayer. At all depths, the angle of the hydrophobic moment of the toxin (with respect to the bilayer normal) fluctuated within a range of <45° about its starting angle, suggesting that the native orientation of SGTx1 in a bilayer membrane is such that its hydrophilic face sits in the interfacial region and its hydrophobic face is exposed to the bilayer core.

For SGTX-PC-CG, in each of the three simulations the toxin diffuses in the aqueous phase for ~30 ns before partitioning into the membrane at a distance of 23–24 Å from the bilayer center. For SGTX-PEPG-CG, partitioning occurred somewhat faster (within ~5 ns), to a distance of 25–26 Å (Fig. 3). The difference in the duration of time before partitioning could be explained if one considers that the positively charged toxin (overall charge of +3) can be expected to form stronger interactions with the anionic interfacial region of the POPE/POPG bilayer. Both depths correspond to the membrane/water interface of the bilayer, which correlates well with the results of the atomistic simulations. The angle of the hydrophobic moment of the toxin in both simulations stabilizes at an average of ~125°, which corresponds to the hydrophobic face of SGTx1 being exposed to the lipid tails. Postpartitioning, SGTx1 exhibited a degree of lateral drift along the plane of the bilayer, with the lipid molecules dynamically repacking around the toxin. The toxin remained at this interfacial location for the remainder of the simulation, suggesting that it is in a stable configuration.

It is important to characterize the interactions that govern the stability of this particular system. In Fig. 4, we analyze the average (over the final 150 ns of simulation) particle densities of the charged components of the systems for SGTX-PC-CG and SGTX-PEPG-CG. We see a high degree of overlap of the basic residues of the toxin with the phosphate group of the lipid molecules, and of the acidic residues with the choline moiety of POPC and the ethanolamine moiety of

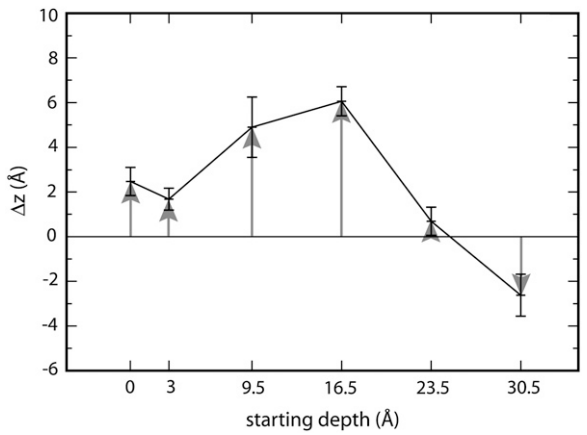

FIGURE 2 Average displacement of the toxin from the different toxin starting depths during the course of the SGTX-PEPG simulation. Bars represent standard deviation. Gray arrows indicate the directionality of movement of the toxin relative to the bilayer.

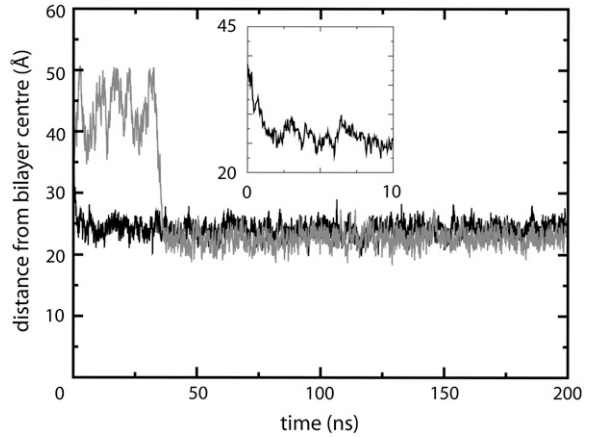

FIGURE 3 Distance of toxin center of mass from bilayer center of mass measured along the bilayer normal for the coarse-grained simulations; SGTX-PC-CG (*dark gray*) and SGTX-PEPG-CG (*black*). The inset is for SGTX-PEPG-CG, shown over a 10 ns duration.

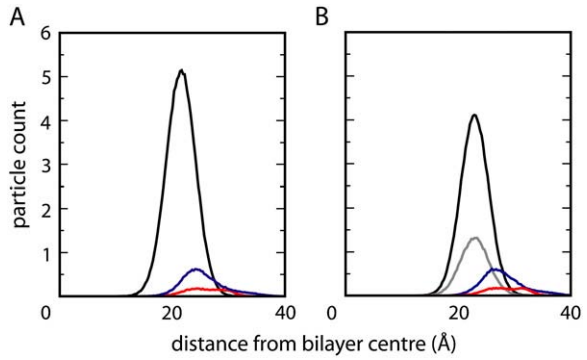

FIGURE 4 Distribution of lipid headgroup and toxin charged groups at the membrane/water interface for simulations (*A*) SGTX-PC-CG and (*B*) SGTX-PEPG-CG, both from *t* = 50 to 200 ns. In *A*, the black line shows the distribution of the phosphates of POPC. In *B*, the phosphate of POPE (*black*) and POPG (*gray*) are shown separately. The distributions of basic and acidic residues are in blue and red, respectively.

POPE. Analyses of the atomistic systems (SGTX-PC and SGTX-PEPG) reveal an increasing trend in the number of contacts (using a cutoff of 4 Å) between the six basic residues of toxin and the lipid phosphates over the simulation period (data not shown). These results suggest that electrostatic interactions are likely to play an important role in stabilizing the toxin at a particular depth within a bilayer membrane.

Given the important role of water in many biological systems, it would not be surprising for water molecules to play a role in stabilizing the toxin in the membrane. Our atomistic simulations reveal that water is able to penetrate the headgroup region of the bilayer. The interfacial waters form a stabilizing network of H-bonds with the toxin. The toxin is additionally able to form H-bonds with phosphates and carbonyls of the POPC, POPE, and POPG lipids and with the ethanolamine and glycerol moieties of POPE and POPG, respectively. Our simulations suggest that SGTx1 preferentially forms H-bonds with the lipids over the water molecules. Investigation of the potential energies of the system (data not shown) reveal a dynamic interplay between the toxin-lipid and toxin-water interactions that determine the toxin preferred depths in the bilayer.

Our results demonstrate that SGTx1 is able to partition into a bilayer membrane, where it stabilizes at the membrane/water interface. This is consistent with previous simulations of VSTx1 with lipid bilayer membranes (11). This behavior of SGTx1 (and other gating modifier toxins) is due to its distinct molecular architecture, which most probably is instrumental in its role as a gating-modifier toxin. It is interesting to relate our results to the available structures of Kv channels and the proposed mechanisms of gating. SGTx1 has been shown to stabilize the closed state of Kv2.1 (5). Our results suggest if membrane partitioning is involved in the mechanism by which SGTx1 inhibits Kv2.1, binding of SGTx1 to the S3b-S4a region of the VS of Kv2.1 is likely to occur at the membrane/water interface. This suggests that the S3b-S4a region of the VS of Kv2.1 may be located in close proximity to the extracellular membrane/water interface, at least when the channel is a (closed) conformation that is able to bind SGTx1. However, this assumes that the local conformation of the lipid bilayer is not greatly perturbed by the VS of the channel, which may not be the case (14). Simulations of a bilayer plus toxin plus Kv channel may provide further insights into the relationship between toxin binding and perturbation of voltage gating.

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