Docking and MD Simulations of the Interaction of the Tarantula Peptide Psalmotoxin-1 with ASIC1a Channels Using a Homology Model

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The interaction of the tarantula toxin PcTx1 with the hASIC1a ion channel is investigated here along homology modeling (using the crystal structure of the cASIC1 channel as a template and the known sequence of hASIC1a), automated docking (using the NMR solution structure of PcTx1), and molecular dynamics simulations (taking into account proton-binding sites), in what represents the first modeling and computational chemistry in the whole family of ASIC/DEG/ENaCs/FaNaCh channels. The results agree with binding and electrophysiological data for the interaction of mutant ^{125}I-PcTx1Y_N with rASIC1a chimeras and PcTx1 itself with hASIC1a chimeras. They go even farther by revealing that only two hASIC1a subunits can be directly involved in the binding, to which four domains - instead of the only two identified by the experiments - participate. Mapping the closest lying amino acids of the homology model and PcTx1 can have heuristic value in stimulating ideas, software, and experimentation.

INTRODUCTION

Acid-sensing ASIC channels are selective, cation-permeable, ligand-gated ion channels, where the activating ligands are extracellular protons. As far as the sequence of amino acids is concerned, they are related to degenerin channels (DEG), epithelial sodium cation (ENaCs) channels, and FMRF-amide peptide-gated channels (FaNaCh).[1]

The extracellular portion of ASIC channels is far larger and intricate that the transmembrane region.

This arrangement provides an assortment of receptors which opens wide prospects for sensory detection, so that ASIC channels are under intense scrutiny for their ability in sensing proton gradients.[2,3] Inhibitors of ASIC channels may reduce epilepsy- and ischemia-driven brain injury,[4] as epilepsy episodes induce significant extracellular acidification.[5] Two types of inhibitors are known. The first type involves binding within the pore, blocking the transit of cations, such as it occurs with most ASICs by a diuretic synthetic chlorinated pyrazine derivative agent called amiloride.[1] With the second type of blockers, interaction occurs at the extracellular loop.

Psalmotoxin 1 (PcTx1) - a peptide of 40 amino acids isolated from the venom of the aggressive Trinidad chevron tarantula (*Psalmopoeus cambridgei*)[6] - is a second-type inhibitor of ASIC1a in either the open or desensitized state,[7] where it is able to extend the range of proton sensitivity of the ion channel to higher pH values, up to pH 7.4,[8] while promoting desensitization.[7] PcTx1, other than a specific blocker of ASIC1a,[9] has been recently shown to interact with ASIC1b[10] too, in this case promoting its opening under slightly acidic conditions.[7,11]

A prediction that the second type of ligands may offer a wider chance than pore blockers of finding clinically acceptable blockers of ASIC channels[9] is materializing. Thus, from studies with rats, PcTx1 has been recently shown to possess opioid-type analgesic properties via ASIC1a channels.[12] This means that central inhibition of ASIC1a, acting upstream of the opiate system, might be exploited to treat any type of pain. PcTx1, being nonlethal, is thus a promising candidate for nonoral therapeutic use.[12] Also, APETx2, a 42-amino acids peptide isolated from the sea anemone *Anthopleura elegantissima*, shows powerful analgesic effects against primary inflammation-induced hyperalgesia in rats via heteromeric ASIC3 channels, while ASIC1a channels are not involved.[13] Therefore, inhibition of the ASIC3 channel by APETx2 offers opportunities for local treatment of inflammation.[13]

Structural details for interactions by PcTx1 have been investigated for both the rASIC1a channel, by making recourse to chimeras while exploiting binding and electrophysiological experiments,[9] and hASIC1a channel chimeras with electrophysiological experiments alone.[7] Now, with the publication of the X-ray diffraction structure of cASIC1 at 1.9 Å resolution,[14] and the elucidation of the solution structure of PcTx1 by two-dimensional ^{1}H NMR spectra,[15] it has become possible to investigate by theoretical methods the interaction of PcTx1 with ASIC1a channels. Relying on the high degree of sequence similarity for these channels, it was chosen here to build the homology model hASIC1a as most relevant to human health problems.

COMPUTATIONAL METHODS

Computations were carried out at 64bit, using a parallel machine, driven by Debian Linux amd64 lenny.

Homology Modeling. The 3D homology model of the hASIC1a channel was built, using the computer program MODELLER 9v4,[16] from the 1.9 Å crystal structure of the homotrimer cASIC1 ion channel (PDB accession code 2QTS) and the known sequence of hASIC1a.[14] Nearly the whole crystallographic data for the protein were retained,

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including its chloride ion ligands, while all lipids, carbohydrates, and water molecules were discarded. Chloride ion ligands were treated as pointlike (BLK) residues, relying on the conservation of the distances from its ligands by MODELLER. The structure for chains A-C was started from amino acid 42, adding two undefined places and ending at amino acids 461 and 457 for chains B and C, respectively. Bearing in mind that the intracellular termini are disordered,[14] the structures of chains A and B were ended at amino acid 463 (with undefined positions 459−463) and amino acid 461 (with undefined positions 459−461), respectively. The undefined positions were filled automatically by MODELLER from the hASIC1a sequence. Neither histidine nor aspartate protonation was manually imposed, the choice being left to the software REDUCE.[17] Twenty-five models were generated, and the one with the best DOPE assessment score was selected. The pore region was embedded into a membrane made with POPC molecules (1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine) hydrated at the polar head. The whole was hydrated with TIP3BOX water in an octahedral box, minimized, brought gradually to 300 K, and subjected to molecular dynamics (MD) simulations for 1 ns in order to alleviate any possible crystallographic constraint.

Docking. The homology model above, freed of water and POPC residues, was used as a stationary molecule to simulate docking by PcTx1 (PDB accession code 1LMM)[15] as the moving molecule. The vdw for chloride ion was set to 1.80 Å. Automated docking was performed using the DOT 2.0 software,[18] which calculates the partial atomic charges at heavy atoms and polar hydrogens for the moving molecule and uses external software to accomplish other tasks, as follows. Addition of hydrogens to both the stationary and the moving molecule is performed with the software REDUCE.[17] The software MSMS[19] is used to describe the shape of the stationary molecule by an excluded volume surrounded by a 3 Å favorable layer, by rolling a probe sphere of radius 1.4 Å. The software APBS[20] is used to calculate the electrostatic potential of the stationary molecule at heavy atoms and polar hydrogens, restricted to the solvent excluded surface. In the present study, this required a grid size as large as 384 Å, to ensure that - under the periodic conditions of work by DOT - the relatively small toxin PcTx1, when close to such a large molecule as the hASIC1a homology model, was not influenced by its shape or electrostatic properties in adjacent cells. Grid space was set to 1 Å. Because no details at atomic or amino acid level were available for the interaction of either the monoiodinated $PcTx1Y_N$ variant (^{125}I- $PcTx1Y_N$, bearing an extra tyrosine residue at the amino terminal) with rASIC1a chimeras,[9] or PcTx1 with hASIC1a chimeras,[7] it was not possible to set restraints in the simulated docking, unlike in a recent study of isoindolinone inhibitors of the MDM2-p53 interaction.[21] However, both before and after automated docking, the interacting molecules and the environment were adapted to the structural features of the template cASIC1[14] and - to the extent that the computational burden remained manageable - the experimental conditions of the electrophysiological and binding studies.[7,9]

Molecular Dynamics. While PcTx1 in the complex was left at its native status of protonation,[6,15] the homology model hASIC1a in the complex was manually monoprotonated at the carboxyl-carboxylate couples indicated by Jasti et al.[14] as putative proton binding sites. Building of a 80 × 80 Å POPC membrane, and embedding the hASIC1a-PcTx1 complex into it, was accomplished by the methodology previously described in detail for the complex of a ladder polyether with a potassium channel homology model.[22] The ensemble was neutralized with sodium ions, which were automatically positioned by the software LEaP,[23] using a Coulombic potential on the computed grid. The ensemble was thus composed, besides the hASIC1a-Pctx1 complex, of 111 molecules of POPC, 18 sodium ions, and 58,685 molecules of TIP3BOX water in an octahedral box. MD simulations were performed using the software AMBER 10[23] with force fields ff99SB for the standard amino acids and GAFF for the POPC molecules.[22] The hASIC1a-PcTx1 complex, with the pore region embedded into a POPC membrane, was first subjected to minimization by steepest descent for 20,000 steps with no restraints, in order to remove any possible steric clash, followed by minimization by conjugated gradient for 10,000 steps, all at constant volume, with no restraints. The resulting ensemble was heated gradually from 0.1 to 300 K in 25,000 steps with restraints on the complex, polar heads of POPC as well as the chloride ions and their ligands, which were chosen according to indications by the X-ray diffraction data.[14] This was followed by equilibration at 300 K with gradual removal of restraints, except those on the chloride ions and their ligands, during 50,000 steps, followed by 1.6 ns production. Restraints on chloride ions ligands were needed in the absence of an ionic concentration to replace them once they leave, keeping in mind that, during experimental observations, the one chloride ion that one sees in a place is not always the same. All steps after minimization were carried out with 0.002 ps time-step. Restraints were by a force constant of 32 kcal/(mol Å^2), and SHAKE was used. Analysis of the trajectories was carried out with the software RMSD TRAJECTORY TOOL and CLUSTER.[24] Mapping of the residues around the PcTx1 ligand as well as all graphics were obtained with the software CHIMERA,[25] version 1.2480.

RESULTS AND DISCUSSION

Binding of the tarantula peptide psalmotoxin 1 (PcTx1) to the hASIC1a channel was investigated here by homology modeling of the channel, automated docking, and molecular dynamics simulations. PcTx1 is known to inhibit specifically ASIC1a currents. From binding experiments with a monoiodinated variant bearing an extra tyrosine residue at the amino terminal, ^{125}I-$PcTx1Y_N$, and electrophysiological experiments with rASIC1a chimeras at pH 7.25 (which should correspond to the desensitized state of the channel[7]), it has been concluded that PcTx1 inserts between what have been called domain 3 (amino acids 158−187) and domain 5 (amino acids 271−369) on the extracellular loop, rather than occluding directly the pore.[9] A shorter stretch of domain 3 (amino acids 167−185) has been identified from electrophysiological experiments to determine high affinity of PcTx1 for the desensitized state of hASIC1a.[7]

Homology Modeling. The homology model of hASIC1a is structurally nearly identical to the template (the crystal structure of cASIC1, apparently in the desensitized state,[26] PDB accession code 2QTS), as a result of a very high sequence identity. Structural analogy includes the fenestrations near the extracellular membrane surface and the

markedly different conformations of the transmembrane domains, as described for the template.[14] Also for rASIC1a[9] the sequence is very similar. The structure of the original cASIC1 construct[14] was retained in the hASIC1a homology model, including the chloride ion ligands, while crystallization water, lipids, and carbohydrates were removed. Protonation was left to the smart software REDUCE, without acting directly on the histidine residues and the ASP residues that lie close to other ASP or to GLU, as indicated by Jasti et al.[14] All attempts at evaluating pK_a values for this complex system with ad hoc software proved largely disappointing. At any event, neither these residues, nor histidine, lie at binding range from PcTx1. The homology model, built with MODELLER, proved free from steric clashes; this notwithstanding, it was minimized and subjected to MD simulation under conditions that approach the binding and electrophysiological experiments.[7,9] To this end, the pore region was embedded into a POPC membrane, and the whole was solvated with water. The temperature was gradually risen to 300 K. This should ensure that any constraints imposed by the crystal state to the template were removed.

Docking and Molecular Dynamics. Automated docking with the software DOT was carried out at its highest level, which involves a 6 degrees orientational search. This resulted in 54,000 placements, which were chosen on the basis of electrostatic plus van der Waals terms. The 30 top ranking placements, evaluated also on the basis of pairwise atomic contact energy, were practically superimposable to the averaged placement. All of them gave the same mapping of amino acids around PcTx1. The first top-placement complex was protonated at all histidine residues and the aspartate residues that lie close to another aspartate or glutamate, as indicated by the X-ray diffraction data for cASIC1.[14] That was simply accomplished by replacing HIS (or HIE) residue names with HIP, and ASP residue name with ASH, in the PDB file. The ensemble was subjected to MD simulations, as described above. The results are illustrated in Figures 1−3, taken from the snapshot corresponding to the potential energy minimum for the ensemble.

The general architecture of the complex, deprived of water and sodium ions for clarity, can be appreciated from Figure 1, where the ensemble backbone is viewed parallel to the POPC membrane plane (for a corresponding stereoview, see Figure S1 in the Supporting Information). It is seen that the peptide PcTx1, shown in red, inserts between four structural motifs. Two are in accordance with experimental data. Thus, highlighted in green is the stretch of amino acids 167−185 identified by Chen et al.[7] for hASIC1a-PcTx1 on the basis of electrophysiological experiments with a series of chimeras. Domain 3 identified by Salinas et al.[9] on the basis of binding and electrophysiological experiments for rASIC1a-^{125}I-PcTx1Y$_N$ using a series of chimeras is longer, comprising the amino acids 158−166. The short helix of exceeding 158−166 amino acids is shown on the upper extreme right of Figure 1, highlighted in forest green. On the upper left side of Figure 1, domain 5 (amino acids 271−369), identified by Salinas et al.[9] for rASIC1a-^{125}I-PcTx1Y$_N$ from binding and electrophysiological experiments with a series of chimeras, is highlighted in blue.

The other two structural motifs that, according to present simulations, make part of the putative binding site of PcTx1 are highlighted in orange (amino acids 113−129, corresponding to Salinas's[9] domain 2 and Jasti's[14] domain α1) and magenta (amino acid 219, which makes part of Salinas's[9] domain 4, corresponding to Jasti's[14] domain β6).

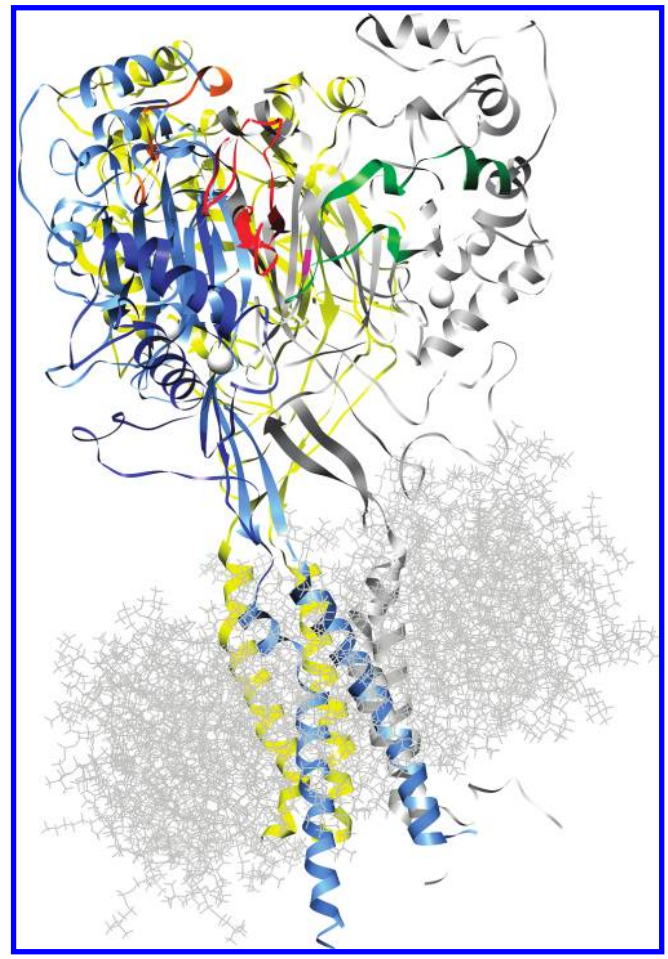

Figure 1. Structure of the hASIC1a-PcTx1 complex resulting from homology modeling, automated docking, and MD simulation, viewed parallel to the POPC membrane plane. Subunits A, B, and C are highlighted in gray, cornflower blue, and yellow, respectively. Water molecules and neutralizing sodium ions were hidden for clarity, while chloride ions, one for each subunit, are seen as gray balls. A POPC membrane (highlighted in gray) surrounds the pore region. The tarantula toxin PcTx1, highlighted in red, was found at less than 5 Å from residues of the green and magenta portions of chain A, to the right, and the blue and orange portions of chain B, to the left. This matches experimental findings for the green portion (amino acids 167−185, as identified by Chen et al.[7] for the hASIC1a-PcTx1 complex and Salinas et al.[9] for the rASIC1a-^{125}I-PcTx1Y$_N$ complex) and the blue portion (amino acids 271−369, identified as domain 5 by Salinas et al.[8] for the rASIC1a-^{125}I-PcTx1Y$_N$ complex). The forest-green highlighted portion of domain 3 (amino acids 158−166, identified by Salinas et al.[8] for the rASIC1a-^{125}I-PcTx1Y$_N$ complex) lies outside the binding range. The portion highlighted in magenta (residue Glu 219 of subunit A) makes part of Salinas's[9] domain 4 (= Jasti's[14] domain β6), while the one in orange belongs to Salinas's[9] domain 2 (= Jasti's[14] domain α1).

It should be noticed that the orange and magenta motifs belong to subunit A, otherwise highlighted in gray, while the green and blue motifs belong to subunit B, otherwise highlighted in cornflower blue.

That subunit C, highlighted in yellow, always remains out of range for binding PcTx1 is best appreciated from Figure 2, where the ensemble is viewed parallel to the 3-fold symmetry axis of the model, from the extracellular side (for a corresponding stereoview, see Figure S2 in the Supporting Information). Here, like in Figure 1, the three chloride ions,

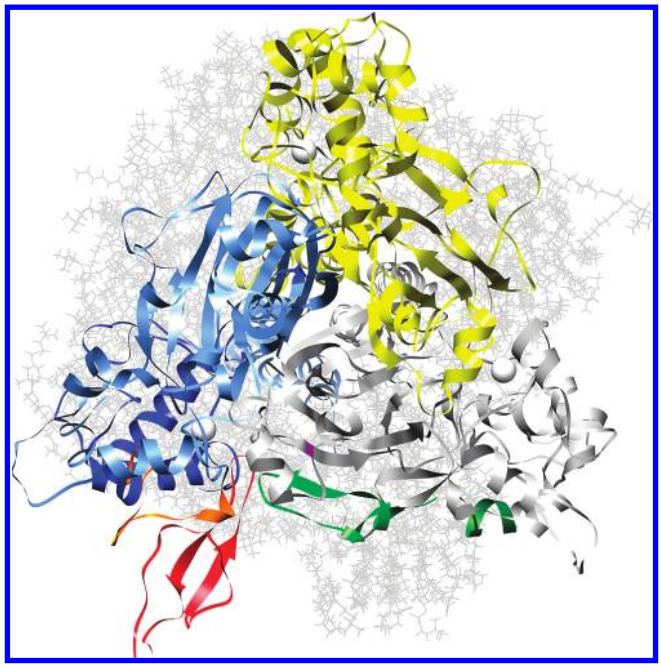

Figure 2. Structure of the hASIC1a-PcTx1complex resulting from homology modeling, automated docking, and MD simulation, viewed parallel to the 3-fold symmetry axis of the model from the extracellular side of the channels. Colors for the component units are the same as in Figure 1. Actually, "three-fold symmetry axis" is somewhat an idealization because the transmembrane helices for subunits A and B are nearly straight, while for subunit C they are substantially bent in cASIC1[14] and hence in this homology model, too, as can be seen from Figure 1.

one for each subunit, are seen as gray balls. Most importantly, the combination of Figures 1 and 2 reveals that domain 3 (green + forest green) and domain 5 (blue) could not be on the same subunit, while, unless PcTx1 binds at the extracellular or intracellular extreme sides of the homotrimer or penetrates the homotrimer, no more than two subunits can be involved in direct binding with PcTx1.

From the same perspective as in Figure 1, the putative binding site of PcTx1 can be seen in greater details in Figure 3a, while Figure 3b offers a complementary side view, where the hASIC1a residues making the putative PcTx1 binding site are only shown (for corresponding stereoviews, see Figure S3a and S3b, respectively, in the Supporting Information). Selected hASIC1a and PcTx1 residues, which are separated by less that 5 Å, are labeled. The full list of residues which meet this spatial criterion is seen in Table 1.

Superimposing the free form of PcTx1[15] (used as input for docking onto the hASIC1a homology model) to the form resulting from docking and MD simulation in a hydrated membrane reveals that some reshaping has occurred for the starting and ending portions of the peptide. This can be best appreciated from Figure S4a (or S4c in stereo) and S4b (or S4d in stereo) in the Supporting Information, from the same viewpoints as for Figure 3a and 3b, respectively.

CONCLUSIONS

Here it is reported on homology modeling of the desensitized state of the hASIC1a channel, automated docking of the tarantula peptide PcTx1, and molecular dynamics of their complex. The 1.9 Å crystal structure of the closely related chicken ASIC1 channel[14] was used as a template for the

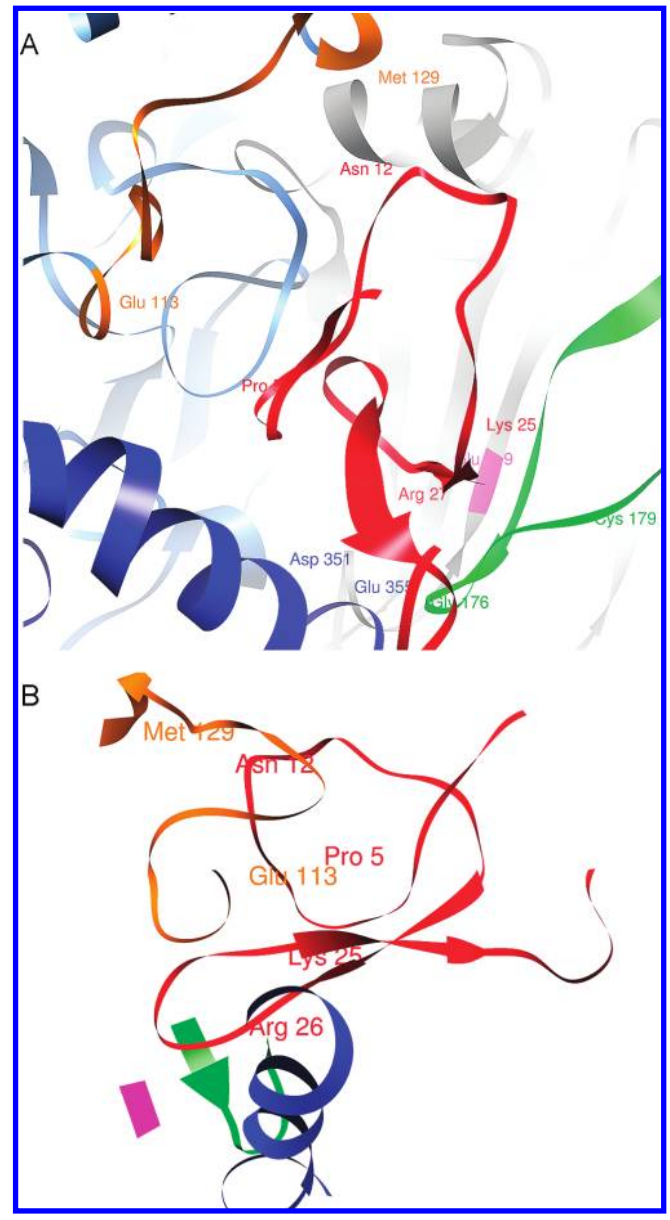

Figure 3. a. Surface view of the putative PcTx1-binding site, from the same orientation, and with the same colors, as in Figure 1. Selected residues for the four stretches composing the putative PcTx1 are labeled, except Glu 219, shown in magenta. The intercorrespondence between residues separated by less than 5 Å is given in the same rows of Table 1, where all residues fulfilling this criterion are listed. b. Same as part a, by turning the ensemble by about 90 degrees clockwise around the model 3-fold axis (vertical axis in the plane of paper or screen) and eliminating all residues of hASIC1a, except those making the putative PcTx1 binding site. Colors as in part a, while only selected residues of PcTx1 are labeled.

homology modeling. To my knowledge, this is the first example of modeling and computational chemistry in the whole family of ASIC/DEG/ENaCs/FaNaCh channels. Automated docking of the 40 amino acids peptide PcTx1 - a known specific blocker of ASIC1a channels - was carried out with this 3D model using the NMR solution structure of the peptide.[15] The pore region of the top-placement complex was embedded into a bilayer POPC membrane, hydrated, and refined by molecular dynamics simulations. The results of these simulations are in a general agreement with electrophysiological experiments using PcTx1 and hASIC1a chimeras[7] as well as binding and electrophysiological experiments using a monoiodinated variant of

Table 1. Mapping, from the Least-Energy Ensemble, of hASIC1a Homology-Model Residues Separated by Less than 5 Å from Tarantula Peptide PcTx1 Residues[a]

hASIC1a chain A[b]	hASIC1a chain B[b]	PcTx1
	Glu 113	Lys 8, Pro 5
	Leu 116	Val 11, Phe 30
	Asn 119	Lys 8, Val 11, Asn 12
	Asn 120	Ile 4, Pro 5, Lys 8
	Arg 121	Asp 2, Cys 3
	Pro 125	Asn 12
	Asp 126	Asn 12, Arg 13
	Met 129	Asn 12, Hie 14
Cys 172		Arg 27
Phe 174		Lys 25, Arg 26, Arg 27
Arg 175		Arg 27
Gly 176		Arg 27
Glu 177		Arg 27
Val 178		Arg 27
Cys 179		Lys 25
Glu 219		Arg 27, Arg 28
	Lys 343	Trp 7, Lys 8, Gly 9, Phe 30
	Glu 344	Lys 6, Trp 7
	Cys 345	Trp 7
	Asn 347	Arg 26
	Pro 348	Trp 7
	Leu 350	Arg 26
	Asp 351	Arg 26
	Glu 355	Arg 26, Arg 27
	Lys 356	Trp 24, Arg 26

[a] Residues that fulfill the title spatial requirement are listed on the same row. [b] hASIC1a numbering.

PcTx1 and rASIC1a.[9] The computer simulations, while revealing that only two subunits of hASIC1a are directly involved in binding PcTx1, show a more strict encapsulation of PcTx1 than was previously thought. According to the theory used in the simulations, Figure 3 shows that binding PcTx1 also involves stretches of both Salinas's[9] domain 2 (which corresponds to Jasti's[14] domain α1) and Salinas et al.[9] domain 4 (corresponding to Jasti's[14] domain β6). Possibly, these two extra domains did not emerge from electrophysiological and binding experimentation[7,9] because of low affinity under the conditions of the experiments, as it was already suspected for domain 4.[9]

The insight gained from these simulations is not only synergistic with the experimental results[7,9] but also clarifies aspects that may be experimentally elusive, such as, first, that the two binding domains of ASIC1a[8] cannot be on the same subunit, second, that only two subunits may become involved, and, third, that PcTx1 may be more strictly encapsulated into hASIC1a than previously thought. Much remains to be done from the computational side for this highly complex system with multiform sites of protonations and state-dependent behavior.[7] Future availability of both experimentally derived restraints, to be imposed to docking and MD simulations like it was recently done with isoindolinone inhibitors of the MDM2-p53 interaction,[21] and code for MD simulations at constant pH in explicit medium will foster advances. Until it will become possible to unravel the time dependency of the structural motifs of ASIC1a, that is how these channels work. Immediately, this work paves the way to in silico search for improved peptides, for example, ones that, while blocking ASIC1a channels, are adapted to oral administration.

Supporting Information Available: Stereoviews corresponding to Figures 1−3 as well as mono- and stereoviews of the matching of the starting NMR conformation and end conformation (after docking and MD simulation) of PcTx1. This material is available free of charge via the Internet at http://pubs.acs.org/.

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