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ARTICLE

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Molecular simulation of the interaction of κ-conotoxin-PVIIA with the Shaker potassium channel pore

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Abstract Molecular simulation techniques were applied to predict the interaction of the voltage-dependent Shaker potassium channel with the channel-blocking toxin κ-conotoxin-PVIIA (PVIIA). A structural three-dimensional model of the extracellular vestibule of the potassium channel was constructed based on structural homologies with the bacterial potassium channel Kcsa, whose structure has been solved by X-ray crystallography. The docking of the PVIIA molecule was obtained by a geometric recognition algorithm, yielding 100 possible conformations. A series of residue-residue distance restraints, predicted from mutation-cycle experiments, were used to select a small set of a plausible channel-toxin complex models among the resulting possible conformations. The four final conformations, with similar characteristics, can explain most of the single-point mutation experiments done with this system. The models of the Shaker-PVIIA interaction predict two clusters of amino acids, critical for the binding of the toxin to the channel. The first cluster is the amino acids R2, I3, Q6 and K7 that form the plug of the toxin that interacts with the entrance to the selectivity filter of the channel. The second cluster of residues, R22, F23, N24 and K25, interacts with a channel region near to the external entrance of the pore vestibule. The consistency of the obtained models and the experimental data indicate that the Shaker-PVIIA complex model is reasonable and can be used in further biological studies such as the rational design of blocking agents of potassium channels and the mutagenesis of both toxins and potassium channels.

Keywords Protein-protein interaction · Potassium channel · Molecular modeling · Conotoxin

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Introduction

The venom of predator marine snails of the genus *Conus*, composed of ~500 species, contains a mixture of small peptides (conotoxins), targeted to specific isoforms of receptor or ion channels (Olivera 1997). κ-conotoxin-PVIIA (PVIIA) is a 27-residue peptide component of the *Conus purpurascens* venom found to inhibit voltage-activated potassium channels. This peptide acts rapidly on its target, so is proposed to have an important role in quick exocitotoxic prey immobilization after the venomous sting (Olivera 1997; Terlau et al. 1996). Its structure has been solved by multidimensional NMR techniques by two groups (Savarin et al. 1988; Scanlon et al. 1997), and two mechanisms of binding of the toxin to the channel have been hypothesized. Scanlon et al. (1997) proposed a strong analogy between PVIIA and charybdotoxin, suggesting that the amino acids H11, R18, K19 and R22 may correspond to the four key residues of charybdotoxin, K11, R25, L27 and R31. Conversely, Terlau and collaborators, based on a series of single-point mutation experiments, concluded that the residues R2, K7, F9 and K25 are determinants for the toxin binding to the Shaker channel (Jacobsen et al. 2000). Furthermore, Terlau and co-workers extended their study by a series of mutation-cycle experiments, providing information to clarify the specific interaction of PVIIA and the external vestibule of the Shaker channel pore.

Since the report of the three-dimensional structure of the bacterial potassium channel Kcsa (Doyle et al. 1998), several groups have initiated an intensive investigation into the permeation of ions through the pore using molecular dynamics techniques (Allen et al. 2000; Aqvist and Luzhkov 2000; Cui et al. 2001; Guidoni et al. 1999), as well as simulations of the structure of the pore region of other channels, like mammalian potassium channels (Rauer et al. 2000; Wrisch and Grissmer 2000), sodium channels (Dudley et al. 2000; Lipkind and Fozzard 2000; Penzotti et al. 2001) and calcium channels (Corry et al.

2000). Interestingly, the initial proposal of work devoted to the simulation of the sodium and potassium channel structures was the study of the interaction of the channel with toxins, as they constitute an excellent molecular probe of the channel structure and may have pharmaceutical interest. In fact, the iterative use of modeling techniques and functional analysis may result in designed molecules to target specific channels (Rauer et al. 2000).

In the work described in this paper we modeled the interaction of PVIIA with the external vestibule of the voltage-dependent potassium Shaker channel. The Shaker channel model was constructed by homology to the three-dimensional structure of the bacterial inward rectifier potassium channel Kcsa, and the binding was simulated by a geometrical docking approach. We used the available experimental data on channel and toxin mutations (Jacobsen et al. 2000) to select the simulation from among the different results, obtaining a model of the Shaker-PVIIA interaction that is compatible with most of the site-specific mutation experiments.

Methods

Homology modeling

The primary structure of the Shaker potassium channel was used to predict the 3D structure of the pore region using Swiss-Model (http://www.expasy.ch/swissmod/), which is a homology modeling server web interface (Guex and Peisch 1997; Peisch 1995, 1996). Further refinements of the predicted structure were made using Swiss-PdbViewer (SPDBV) (Guex and Peisch 1997). Energy minimization was done with the method of steepest decent. Computations were carried out with the GROMOS96 implementation of SPDBV. The evaluation of structural parameters and the prediction quality of the modeled structure were done using the programs WHATIF (Hooft et al. 1996; Vriend 1990) and PROCHECK (Laskowski et al. 1993; Morris et al. 1992) from the Biotech web server (http://biotech.ebi.ac.uk:8400/).

Protein docking

To predict the structure of the channel-toxin complex we applied a geometric recognition algorithm, implemented in the program GRAMM (Katchalski-Katzir et al. 1992). The program performs an exhaustive six-dimensional search through the relative translations and rotations of the molecules. The simulation was done with parameters designed for high-resolution structures (Katchalski-Katzir et al. 1992), and the 100 lowest-energy configurations were analyzed.

Results

Modeling of the Shaker potassium channel pore region

The amino acid sequence of the Shaker channel, cloned from *Drosophila melanogaster* (SwissProt entry P08511), was aligned with the sequence of the *Steptomyces lividans* Kcsa (SwissProt entry Q54397). The segment between H25 and G116 of the Kcsa was aligned with the segment between S392 and Y483 of the Shaker, comprising the transmembrane regions S5 and S6 and the pore region. This alignment, using the BLOSUM62 criteria, yielded 49% of positives, comprising 33% identity (Fig. 1).

The 3D structure of the pore region of the Shaker polypeptide was initially predicted by structural homology using Swiss-Model, making use of the structural information available for Kcsa (PDB entry 1BL8) which has been solved by X-ray diffraction with 3.2 Å resolution (Doyle et al. 1998). The model was constructed by fitting the four subunits of Kcsa with four repeats of the Shaker sequence, imposing the fourfold symmetry of the reference structure to the Shaker model.

Inconsistencies in the initial model were reported as atom clashes and high threading energies by SPDBV. Amino acids presenting such inconsistencies were fixed in successive refinement steps. The side chains of selected amino acids were fixed by choosing the best rotamer by browsing a rotamer library, followed by an intensive cycle of energy minimization, applied to a region around the changed amino acid. When all inconsistencies were solved the model was optimized by applying an energy minimization cycle with the method of steepest decent to the entire model. Calculations of energy minimization were done with a cut-off of 10 Å and using harmonic constraints. The convergence of minimization, considered attained when the total energy variation between successive cycles was less than 0.05 kJ/mol, was –9238 kJ/mol.

The final structure of the predicted Shaker model is similar to that of the Kcsa template (Fig. 2A, B). Differences in the distances of the three backbone atoms, the carboxylic carbon (C), α-carbon (CA) and amino nitrogen (N), of the three chains of the Shaker with respect to the backbone of the Kcsa template are very small, even after the variations produced in the Shaker channel model by the energy minimization procedures (Fig. 2C). The r.m.s. of the position variations are less than 0.25 Å for all four chains, and the r.m.s. value is 0.23 Å (Fig. 3), significantly smaller than the value of 0.5 Å predicted as the r.m.s. deviation between homologous structures.

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                    S5
KCSA      25   HWRAAG AATVLLVIVL LAGSYLAVLA ERGAPGAQLI TYPRALWWSV
                 +  +      + + |  |  +     + + +|  |    | + + ++|+
SHAKER    392  SMRELG LLIFFLFIGV VLFSSAVYFA EAGSENSFFK SIPDAFWWAV

                    P                    S6
KCSA      71   ETATTVGYGD LYPVTLWGRC VAVVVMVAGI TSFGLVTAAL ATWFVG
                + +++++++ | ++ |++|  +  |  |++|  |  +    |  | +
SHAKER    438  VTMTTVGYGD MTPVGVWGKI VGSLCAIAGV LTIALPVPVI VSNFNY
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Fig. 1 Alignment of the primary structure of the pore region of the potassium channels Kcsa, cloned from the bacteria *Steptomyces lividans*, and Shaker, cloned from *Drosophila melanogaster*. The segments of the Shaker fifth transmembrane segment (S5), corresponding to the Kcsa first transmembrane region, the pore-forming region (P) and the sixth transmembrane segment (S6), corresponding to the Kcsa second transmembrane region, are indicated by *wavy lines*. Identical residues are indicated by "+", and other positive matches are indicated by "|"

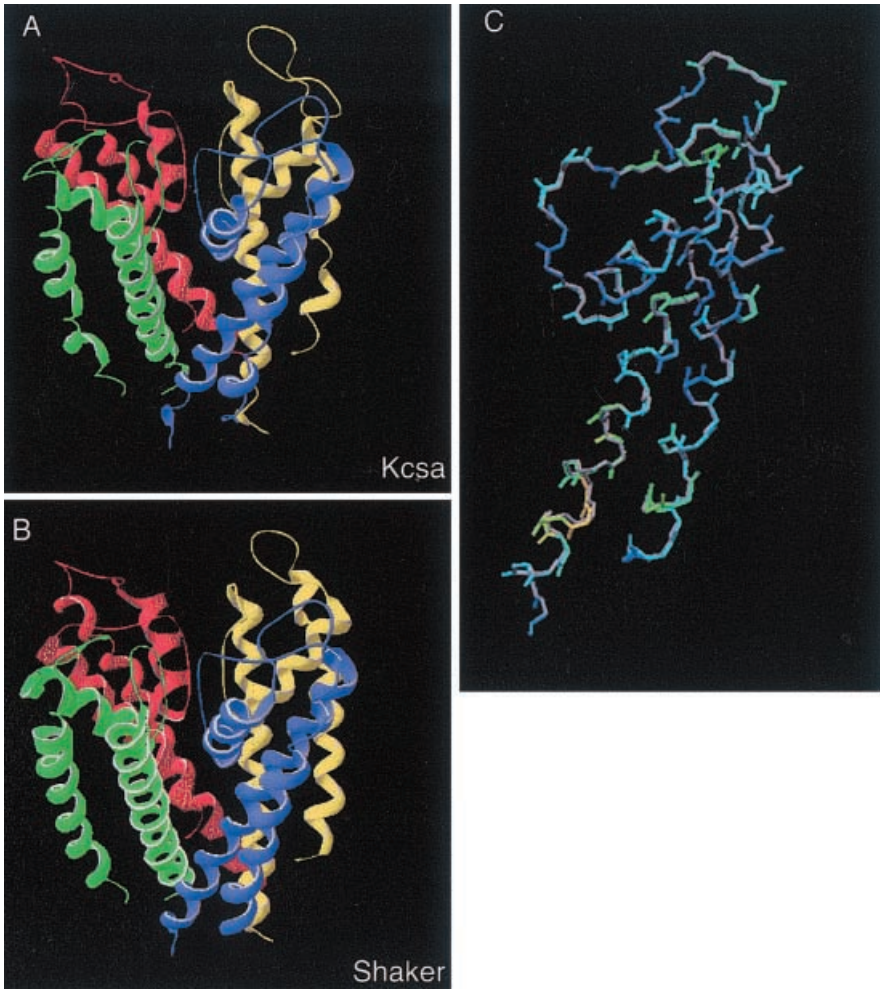

Fig. 2 Comparisons of the three-dimensional structures of the Kcsa channel model, deduced from X-ray diffraction data (**A**), and the Shaker channel model, obtained by homology prediction (**B**). The four chains forming the potassium channels are presented in different colors. Peptidic backbones of one repeat of Kcsa and Shaker are superimposed in **C**. Observe the notable coincidence of the two structures

To evaluate the correctness of the predicted model, a series of quality controls, using the programs WHATIF and PROCHECK, were done. Using the program WHATIF (Hooft et al. 1996; Vriend 1990) for bond angles and lengths, the position-specific rotamer distribution for every residue was determined. Resultant values for the Shaker models conform to the goodness-of-fit criteria defined in the WHATIF program.

The main-chain quality parameters of the Shaker model were evaluated with the program PROCHECK (Laskowski et al. 1993; Morris et al 1992). Evaluation with PROCHECK included the Ramachandran plot, evaluation of the peptide bond planarity, non-bonded interactions, side-chain parameters, and the *G* factor, accounting for the torsion angles and for the covalent geometry, as a measure of the overall "normality" of the structure. All considered parameters analyzed by PROCHECK yielded values that indicated the goodness-of-fit of the predicted structure.

Toxin-Shaker geometrical docking

The docking simulation was done by searching the geometrical interaction of the PVIIA and the external mouth of the Shaker channel model. The PVIIA structure was obtained from the ensemble average of the 20 lowest-energy NMR solutions reported in the PDB database (1AV3). To reduce the amount of computation of the protein docking program, the calculations were done using a fragment of the Shaker model, where the mouth of the channel was defined as the region between residues 419 and 454 of each repeat. The segment of the extracellular pore vestibule used for these calculations is shown in Fig. 4 (panel 0), together with the putative positions of the permeant ions, homologous to those described for Kcsa (Doyle et al. 1998); the amino acids exposed to the external mouth of the channels are colored green. No differences were found when the docking procedure was applied with or without potassium in the most external binding site of the pore, probably because the other geometrical restraints do not allow the toxin to approach close enough to the potassium binding site to "perceive" the presence of the ion.

The docking program GRAMM yielded 100 lowest-energy possible positions for the interaction between the PVIIA and the Shaker fragment used for the simulation. As the calculation performs an exhaustive search for all possible low-energy configurations, without any restraint, a series of improbable configurations were also yielded by

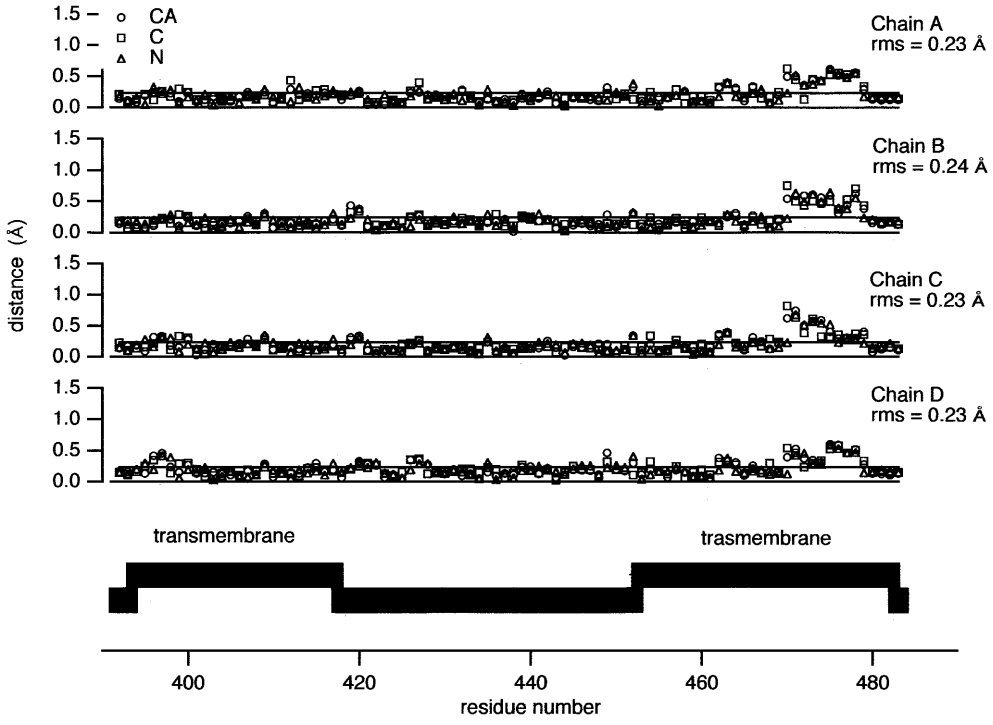

Fig. 3 The deviation of the positions of the backbone for the four chains (A, B, C, D) of the Shaker potassium channel model compared with the Kcsa model. Values represents the distance between the α-carbons (CA, *circles*), carboxylic carbons (C, *squares*) and amino nitrogen (N, *triangles*) between the two structures. The r.m.s. for each chain is indicated in the figure. The *lower panel* indicates the α-helix regions corresponding to the transmembrane segments (the extremes)

the program, as observed by a visual inspection of the Shaker-toxin complexes. There were some models where the toxin evidently was in contact with non-exposed parts of the channel (see Fig. 4, panel 1.1) or the interaction with the channel did not result in an evident obstruction of the pore (see Fig. 4, panels 1.2, 1.3 and 1.4).

Mutation cycle criteria for model selection

The Shaker-PVIIA interaction has been extensively studied by single-point mutation functional experiments (Jacobsen et al. 2000), and data regarding the change in the coupling energy, $\Delta\Delta G$, were reported. The value of $\Delta\Delta G$ obtained on each mutation cycle can be correlated to the physical distance between the two amino acids mutated during the cycle, d_{aa} (Schreiber and Fersht 1995). Values of $\Delta\Delta G$, estimated from affinity data obtained from mutation-cycle experiments on three preparations, barnase-barstar (Schreiber and Fersht 1995) and two antigen-antibody complexes (Dall'Acqua et al. 1996, 1998), were plotted against the d_{aa} values measured for the structural models obtained from X-ray diffraction of crystals of these three complexes (PDB entries 1BRS, 1DVF and 1VFB, respectively). Data were empirically plotted with a simple exponential function (Fig. 5), and the parameters were used to predict the amino acid pair distances, d_p, from the $\Delta\Delta G$ obtained in the Shaker-PVIIA experiments.

To compare the d_{aa} measured in the models and the d_p predicted from the $\Delta\Delta G$ measured in the mutation-cycle experiments, an empirical score criterion was developed. A positive score value was assigned to a given pair when the difference between d_{aa} and d_p was small, and a negative score value, a penalty, was assigned when this difference was large. The final score was calculated as the sum of the partial scores of each amino acid pair. The amino acid pairs were divided into two groups, according to their d_p values, defined as "long distances" and "short distances". The limit value for defining the two groups was 7.5 Å. Changing the limit value between 6.5 Å and 8 Å did not change the results significantly. The score or penalty assigned to each residue pair depended on the distance group, the values being higher for the residue pairs of the short distance group. The parameters used for assigning scores and penalties, as defined below, were defined after testing various combinations of them. The test was done by examining the protein-protein complexes mentioned above. The final score was compared with an expected value, obtained by applying the same score-based comparison procedure to the predicted data with itself. Parameters were changed to obtain the minimal mean difference between the test data and the expected final test score, yielding a mean final score ratio of 0.54.

To examine the channel-toxin complex, we used the following criteria. When, in a given amino acid pair, the distance of the toxin amino acid and at least one amino acid of the four Shaker chains conformed to the condition $|d_{aa}-d_p| < 2$ Å, a score value of $S=1$ was assigned for long distances and a score of $3S$ was assigned for short distances; if the distance conditions were not satisfied for a given short-distance pair, then for each chain when $|d_{aa}-d_p| = 2$, a penalty of $-2.7S$ was assigned; for a long-distance pair, each time that a predicted long-distance pair had $d_{aa} < 7.5$ Å (and a higher $\Delta\Delta G$ would be expected), a penalty of $-0.9S$ was assigned. Using these criteria, a reference final score of 106 was expected if the

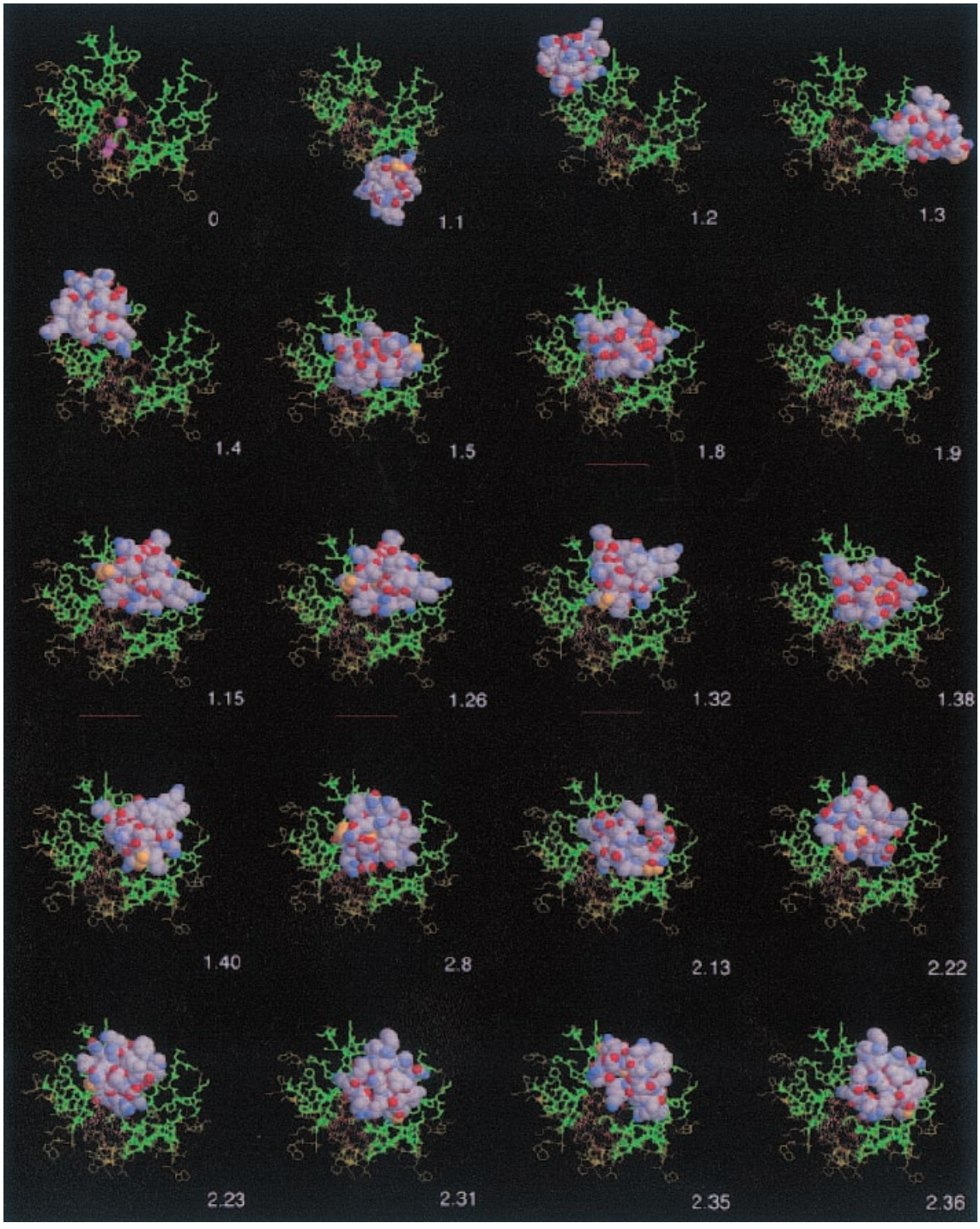

Fig. 4 The extracellular vestibule of the Shaker channel model and 19 of the conformations obtained by the geometrical docking of the PVIIA toxin to the channel. *Numbers* in each panel indicate the identification code of the program results. The model channel fragment used for the docking calculations is shown in panel 0. The exposed residues of the extracellular vestibule that contains the critical amino acids for the channel-toxin interaction are colored *green*, and the selectivity filter is colored *red*. The *pink spheres* indicate permeant ions at the positions equivalent to the Kcsa model. Models indicated by a *red line* (1.8, 1.15, 1.26 and 1.32) are those selected as more plausible models of interaction

predicted distance conditions were satisfied for at least one Shaker chain. This empirical set of criteria allowed us to easily select four models with final scores >60 (test/expected $= 0.56$) for further detailed analysis.

Three-dimensional model for toxin-channel interactions

We compared the characteristics of the resulting four final models, in particular regarding the position of the

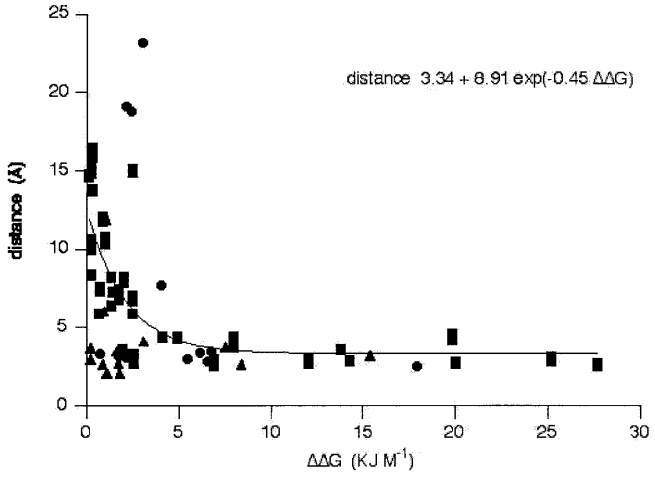

Fig. 5 Change in the coupling energy, $\Delta\Delta G$, estimated from mutation-cycle experiments on barnase-barstar and in two antigen-antibody complexes, are plotted against the amino-acid pair mutated in each cycle and their distance measured from the X-ray diffraction models (PDB entries 1BRS, 1DVF and 1VFB, respectively). The *continuous line* is a single-exponential fit of the data, and the resulted parameters were used to predict the Shaker-toxin distances for model selection

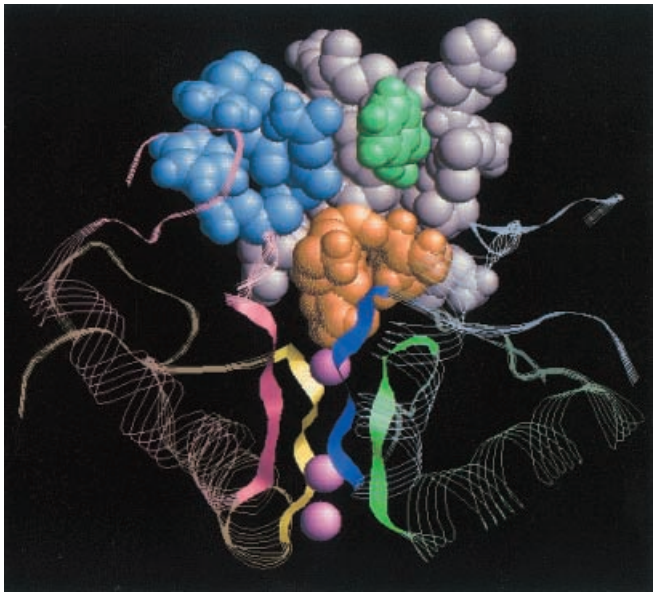

Fig. 6 The model of the pore region of the Shaker channel and the PVIIA toxin. The four subunits of the Shaker channel are presented in different colors, and the region corresponding to the selectivity filter is shown as *solid ribbons*. The three positions of the permeant ions are shown as *pink spheres*. The position of the PVIIA toxin, as predicted by the model 1.8, is shown as space filling. Observe the toxin plug, colored *gold*, which is closely interacting with the entrance of the selectivity filter and with the outer permeant ion. The *sky-blue* region corresponds to the anchoring part of the toxin. The residue F9 is colored *green*

toxin and channel residues that have been described to be critical for binding. Although the relative position of the toxin with respect to the channel external vestibule is not perfectly identical, all four complex models share most of the characteristics expected from the mutation experiments. Therefore, it was not possible to choose between these models, and we considered them all, in principle, as correct. For clarity, we decided to focus on only one model, so the following description concerns model 1.8 (see the corresponding panel in Fig. 4) and the expected interactions between the two polypeptides, as described by single-point mutation experiments in the paper of Terlau's group (Jacobsen et al. 2000). All properties described here are common also for models 1.15, 1.26 and 1.32.

The model toxin-channel interaction is shown in Fig. 6, where the channel extracellular pore vestibule is schematized by ribbons and the toxin is represented as a space-filling model. We have arbitrarily designated the four chains of the Shaker model as A, B, C and D (pink, yellow, green and blue, respectively). The positions of the three permeant ions, equivalent to those described for the Kcsa model, are indicated by space-filling spheres. Two important clusters of amino acids can be well defined in the toxin polypeptide, one forming the toxin plug (colored gold), near to the entrance of the selectivity filter of the channel, and the second representing a more external anchoring region of the protein (colored sky blue). Residue F9, considered as critical for toxin activity (Jacobsen et al. 2000; Savarin et al. 1988), is also indicated in Fig. 6 (colored light green).

The fingerprint TVGYGD (residues 442–446) constitutes the selectivity filter of the Shaker channel, present in most known potassium channels (Doyle et al. 1998). The backbone of these residues constitutes the narrowest part of the channel that accommodates the permeant potassium ions (Doyle et al. 1998). Four amino acids in the toxin, R2, I3, Q6 and K7, are localized in the immediate vicinity of the entrance of the selectivity filter of the channel, and probably constitute the plug of the toxin that impedes the ionic flux. R2 is a very sensitive amino acid of the toxin, and the mutations of this residue to A, Q or K produce a significant reduction of the binding affinity of the toxin. This amino acid is closely interacting with G446 (chain D), at a distance that is consistent with the formation of a hydrogen bond (Fig. 7A). This putative hydrogen bond may be disrupted by the mutations of R2, as in the case of R2A shown in Fig. 7B. Residue I3 is also near to the entry of the selectivity filter, interacting at a longer distance with the channel residues (about 3.5 Å from G446, chain B), in agreement with the relatively smaller effect revealed in the single-point mutation experiments. Mutation-cycle experiments also predict an interaction between I3 and S424, which are more than 5 Å apart. However, the mutation of S424K of chain C, increasing the bulkiness of this residue, produces a decrease of the inter-residue distance to about 2.5 Å (Fig. 7C). Residue Q6 seems to be also involved in the formation of the toxin plug, being close to G446 of chain D, and even is probably interacting directly with the outer permeant ion. Finally, the positively charged residue K7 is situated near to the acidic residue D447 of chain D (Fig. 7A), probably interacting electrostatically. This interaction is lost by the

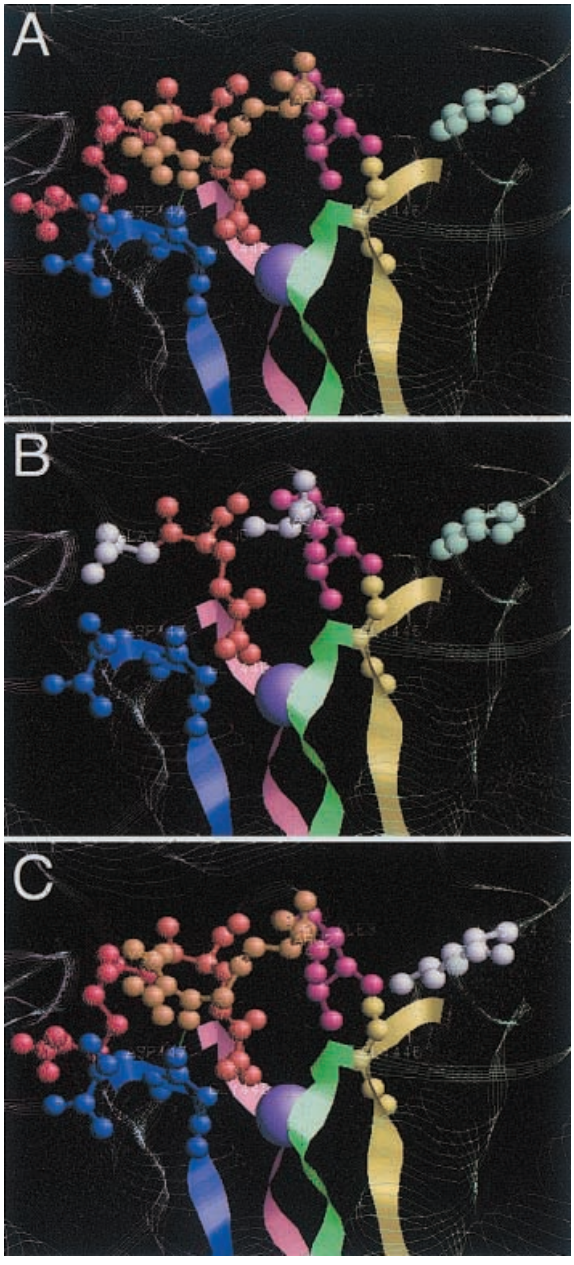

Fig. 7 Close view of the toxin plug region (**A**). The mutation of R2 or K7 to alanine (shown in *white* in **B**) disrupts the close interaction between the toxin and the channel. Differently, the mutation of S424K (colored *white* in **C**) increases the interaction between the channel and I3, probably disturbing the correct docking of PVIIA

mutation K7A, which is reported to reduce significantly the affinity.

The second interesting protein-protein contact region is the cluster of residues of the toxin formed by R22, F23, N24 and K25. The side-chains of this cluster are in the surface of the toxin polypeptide, have a positive net charge, and probably have a high mobility in their side-chains, as revealed by the NMR models. They are situated close to a region of the chain C of the Shaker, formed by the residues E422, N423 and S424 (Fig. 8A). The substitution of any of

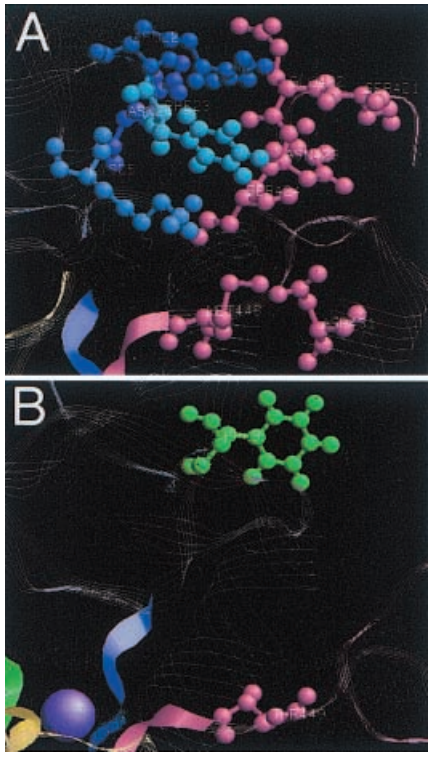

Fig. 8 **A** The anchoring cluster of amino acids of the toxin (colored in *blue tones*) is at a close distance to the channel region, indicated in *pink*. Mutations of any of these toxin or channel amino acids produce consequences for the toxin binding. **B** The toxin residue F9 (*green*) is located far from any other channel amino acid, including T449, that is predicted to be near in the mutation-cycle analysis

these toxin amino acids by A, with consequent neutralization, or a change of any of these channel residues by positively charged K, produces a decrease of the toxin binding affinity, probably because the electrostatic interaction between the two proteins is disturbed. The effect of the mutation S424K is remarkable, and produces some of the maximum changes on toxin binding. These may be caused also by the fact that S424 is quite close to F23 (2.5 Å), as well as by the disturbed interaction of S424 and I3 described above. Also, the charge change produced by the mutation S421K that reduces the toxin affinity, probably determinant for the surface charge of the protein in this region, produces a reduction of the toxin affinity. The channel residues D431 and M448, whose mutations produce subtle effects in the toxin-channel interactions, are predicted as close to K25 by the mutation-cycle experiments. In the case of M448, the short distance to K25 (4.3 Å) may explain these results, but not in the case of D431, at a distance of more than 9 Å. One possibility is that this mutation produces a secondary effect on the structure of the channel that can modify the toxin-channel interactions.

Residue F9 is considered as critical for toxin activity, and from mutation-cycle experiments this residue and Q10 are predicted to closely interact with the channel

amino acid T449. However, none of the models selected show F9 or Q10 in close contact with channel polypeptides (Fig. 8B).

Discussion

We have undertaken the study of the interaction of PVIIA with the external pore vestibule of the voltage-activated potassium Shaker channel. This work had two phases: firstly the construction of a potassium channel model, and secondly the docking of the toxin to the external pore vestibule and the selection of a plausible model that fits with the experimental data. The simulation of the Shaker was restricted to the homologous S5-pore-S6 region of the channel, as it is based on its homology with the bacterial Kcsa channel. The feasibility of the Shaker model obtained was checked using well-established criteria (Hooft et al. 1996; Laskowski et al. 1993; Morris et al. 1992; Vriend 1990), resulting in a set of parameters that indicates a moderately good quality of the simulation. The coincidence of the Shaker model backbone to the Kcsa structure, even after an intensive energy minimization procedure, is remarkably good (see Fig. 2). This confirms that the general features of the Kcsa channel may be applied to the homologous region of other potassium channels, as also concluded for mammalian Ca^{2+}-activated and voltage-activated potassium channels (Rauer et al. 2000; Wrisch and Grissmer 2000), constituting a good starting point for predictions of the interaction of the channel with other molecules.

We used a blind geometrical approach to the toxin docking (Katchalski-Katzir et al. 1992), that resulted in a set of 100 geometrically acceptable conformations for the pore region-toxin interaction, as expected for a system of two molecules with a high degree of freedom (three translations and three rotations). Most of the effort was directed to a reduction of the number of models to a set that can be analyzed. The first inspection of the data, purely based on topological considerations, i.e. that the toxin must bind the extracellular vestibule of the channel occluding the pore, resulted in 24 conformations, still a high number of possible models. For a selection among all conformations obtained, we developed a simple scoring algorithm, based on the predicted distances between the amino acid pairs studied by mutation-cycle experiments (Jacobsen et al. 2000). This algorithm is based on a purely empirical fitting of the change in the coupling energy, $\Delta\Delta G$, and the residue-pair distances observed in proteins where the three-dimensional structure was solved. The parameters of the fitting allowed us to predict the distances between the amino acid pairs based on the experimentally estimated $\Delta\Delta G$ in the Shaker channel-PVIIA system (Jacobsen et al. 2000), and to compare these data with the distances predicted by the 100 obtained conformations. This empirical algorithm yielded four plausible models that share the properties (residue-residue interactions for the Shaker-PVIIA model) which justify most of the functional experiments described for Shaker channels and PVIIA toxin single-point mutants. It is interesting to notice that there are several reports indicating that some peptide toxins which bind the extracellular mouth of the channel do not occlude the pore directly, but exert an electrostatic or steric effect on ion conduction (French and Dudley 1999; Imredy and MacKinnon 2000). However, this seems not to be the case for the PVIIA toxin: the four selected models of Shaker-PVIIA interaction show that the toxin binds the channel occluding the pore.

The proposed model for Shaker channel-PVIIA toxin interaction predicts two regions of the toxin molecule that are critical for the binding. The group of residues R2, I3, Q6 and K7 (colored gold in Fig. 6) have a close interaction with the entrance of the selectivity filter of the channel model, defined by the residues Y445 and G446, and probably also with the outer permeant ion in the channel. Mutations of the residues of this toxin region result in a significant change of the toxin binding affinity (Jacobsen et al. 2000), and probably form the plug of the toxin that impedes the ionic flux through the channel. This close interaction of the toxin with the entrance of the selectivity filter is consistent with the hypothesis of a direct interaction of the permeant potassium ions and the toxin (Garcia et al. 1999; Terlau et al. 1999). The residues R22, F23, N24 and K25 form the second interesting region of the toxin. This group of amino acids confers a partial positive charge to the surface of the PVIIA, and in the models are accommodated near to a channel cluster of amino acids, S421, E422, N423, S424, D431 and M448, located at the surface of the entrance of the extracellular pore vestibule, and with a negative partial charge. This second toxin region may constitute an electrostatic anchorage of the toxin to the channel. The significant changes of the binding affinity produced by single-point mutations of these toxin or channel residues (Jacobsen et al. 2000) are in agreement with this hypothesis. These observations are consistent with the hypothesis of two interaction phases oriented in different directions as a mechanism of binding for conotoxins, named a "Janus-ligand" (Olivera 1997). Potassium channel blocker toxins from different animal sources (Cnidaria, Molusca, Arthropoda, Reptiles), even having a different scaffold, preserve a similar functional dyad, defined by two clusters of residues, including a lysine assisted by a 6.6 ± 1 Å distant aromatic residue (Daulplais et al. 1997). Residues K7 and F23 of PVIIA have been suggested as part of the functional dyad (Jacobsen et al. 2000; Savarin et al. 1988). These two residues, that are at a distance of about 6 Å in the PVIIA toxin, are included in the two binding clusters identified here.

An important drawback of our Shaker-toxin interaction model is the lack of interaction observed for toxin residue F9. A single-point mutation of this residue produces a significant decrease of the binding affinity, and mutation-cycle experiments predict a close interaction of F9 and T449 (Jacobsen et al. 2000). However, we could not observe any interaction between these two residues, located too far apart to justify any strong

interaction (see Fig. 8), and we do not have any explanation for this lack. One possibility is that the PVIIA mutation F9A could produce an inactive folding of the toxin. On the other hand, the models described here are ''frozen'', without considering the mobility of the lateral chains. Preliminary data obtained from molecular dynamics simulations have shown that there are some conformations where the distance between the toxin residue F9 and the channel residue T449 is smaller, as these two residues are located in regions with a relatively high mobility. A more precise explanation for this problem will be probably obtained when the series of molecular dynamics simulations is complete. However, since no more detailed structural information for the Shaker channel is provided, we prefer to remain with a conservative model that can explain most (but not all) of the binding properties studied experimentally.

The last important point is the fact that the set of configurations of the Shaker-toxin complex presented here is a hypothetical model, mostly based on criteria obtained from experimental data, but remains just a working hypothesis that can be useful for future design experiments and for trials for searching for toxin-homologous molecules that may have practical interest.

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