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Implicit two-phase solvation model as a tool to assess conformation and energetics of proteins in membrane-mimetic media

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Abstract. A heterogeneous implicit membrane-mimetic model is applied to simulations of membrane proteins. The model employs atomic solvation parameters for gas–water and gas–cyclohexane transfer. It is used to analyze structure, energetics, and orientation with respect to the bilayer of two polypeptides with different modes of membrane binding – hydrophobic segment of human glycophorin A (GpA) and cytotoxin II from *Naja naja oxiana* snake venom (CTX). The native state of GpA represents a transmembrane (TM) α helix, while CTX is a water-soluble protein, which is able to interact with the cell membrane. The conformational space of the polypeptides was explored in Monte Carlo simulations. The results show that the most stable conformers of GpA represent a TM α helix. They are additionally stabilized by an applied TM voltage. The results also show that CTX inserts with its three loops, does not cross the hydrophobic layer, and stays partially immersed in the membrane. This agrees well with the experimental data, thus confirming the validity of the solvation model.

Key words: Protein conformation – Solvation potential – Molecular modeling – Glycophorin A – Cytotoxin II from *Naja naja oxiana*

1 Introduction

Native conformations of membrane-bound proteins and peptides are determined by the interactions of the peptide chains with each other, with the lipid bilayer, and with water. The prediction of the mechanisms of protein insertion into lipid membranes and its stabilization in the membrane-bound state requires detailed understanding of these interactions. Because of the difficulties in obtaining experimental structural information on membrane proteins, computer simulations become of special interest.

Many theoretical studies [1–5] have been performed on membrane-bound α helices. This is not only because of the simplicity of the system, but also owing to the fact that the membrane-embedded α helices either in transmembrane (TM) or peripheral orientation represent a structural motif which acts autonomously or associates with other peptides forming membrane domains in proteins [6, 7]. Among the structures of membrane-bound peptides, α helices are the most frequently observed; therefore, an understanding of the factors determining the stability of the α-helical fold in a heterogeneous bilayer-mimetic environment is of significant biological relevance. Taken together, this makes the membrane-bound α helix a convenient model to address the questions mentioned earlier. Another membrane-binding spatial motif was found in the family of cardiotoxins: these proteins share a high degree of structural similarity and have a common three-finger β-sheet fold [8]. Cardiotoxins interact with a wide variety of cells – they cause membrane depolarization of nervous and muscle cells and induce lysis of T lymphocytes, erythrocytes, epithelial and other cells [9]. At the same time, the structural aspects of cardiotoxin interaction with membranes remain unclear. It is therefore interesting to understand the molecular events accompanying membrane binding of the two different motifs – α-helix of glycophorin A (GpA) and three-finger β-sheet toxin.

The main difficulty inherent in the application of computational methods to membrane-bound systems is

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related to the correct treatment of protein–lipid interactions. Important progress achieved in molecular dynamics (MD) and Monte Carlo (MC) simulations of explicit hydrated bilayers [10] allows, in principle, studies of the behavior of peptides on the membrane–water interface. At the same time, this approach is too computationally demanding and, therefore, questions about structure and function of membrane proteins can be addressed on relatively short time scales.

An alternative approach lies in the employment of models with implicit consideration of membranes. Such models are of special interest because of their computational efficiency and ability to account for principal trends in protein–lipid interactions. In this approximation, the bilayer is usually treated as a continuum whose properties vary along the membrane thickness, and membrane insertion is simulated using either MC or MD methods [1, 4]. Testing against experimental data shows that the calculations give good predictions both for the association state and the peptide's orientation relative to the membrane surface. Moreover, such models provide a number of insights into the mechanism of the insertion of the peptides into membranes. At the same time, during the simulations the conformation of the peptide is often restrained to an α helix. Hence, the problems related to conformational rearrangements, like formation or destabilization of the secondary structure induced by the environment, cannot be addressed by these techniques.

Recently, we proposed a protein solvation model mimicking polar (water), moderately polar (octanol), and membrane environments [5, 11, 12]. A full-atom protein representation was used, and the conformational space was sampled in unrestrained MC simulations. The method was tested on a number of peptides, and the results were shown to fit the experimental data well. Here we present a more elaborate heterogeneous solvation model based on combined employment of atomic solvation parameters (ASP) for water and hydrocarbons. We describe the application of the model to two polypeptides, which were previously studied in experiments: a TM fragment of human GpA and cytotoxin II (cardiotoxin) from *Naja naja oxiana* snake venom (CTX). These polypeptides demonstrate different modes of binding to cell membranes. GpA is one of the simplest membrane proteins studied so far, and its spatial structure is known at atomic resolution [13]. Its membrane-bound domain represents a single TM α helix. CTX is a 60-residue water-soluble protein, which is also able to interact with membranes and damage a variety of cells [14]. The three-dimensional solution structure of CTX was recently solved in our laboratory [15]. Starting from different orientations with respect to the membrane, the conformational space of these polypeptides was explored in unrestrained MC simulations, and the resulting low-energy states were analyzed in terms of the conformations, energetics, and the mode of membrane binding of the polypeptides. In the case of GpA, the role of the TM voltage was also investigated.

2 Method of calculation

2.1 The membrane model

The potential-energy function was taken in the form $E_{\text{total}} = E_{\text{ECEPP/2}} + E_{\text{solv}} + E_{\Delta V}$. The term $E_{\text{ECEPP/2}}$ includes van der Waals, torsion, electrostatic and hydrogen-bonding contributions [16]. E_{solv} is the solvation energy:

$$E_{\text{solv}} = \sum_{i=1}^{N} \Delta\sigma_i \text{ASP}_i, \tag{1}$$

where $\Delta\sigma_i$ and ASP_i are the accessible surface area (ASA) and the ASP of atom i, and N is the number of atoms. The all-atom protein representation was used. ASP sets imitating both the nonpolar hydrocarbon core of the membrane and aqueous solution were taken from Refs. [5, 11]. The membrane is described as a two-phase system: water/hydrophobic layer/water (Fig. 1), with the following assumptions:

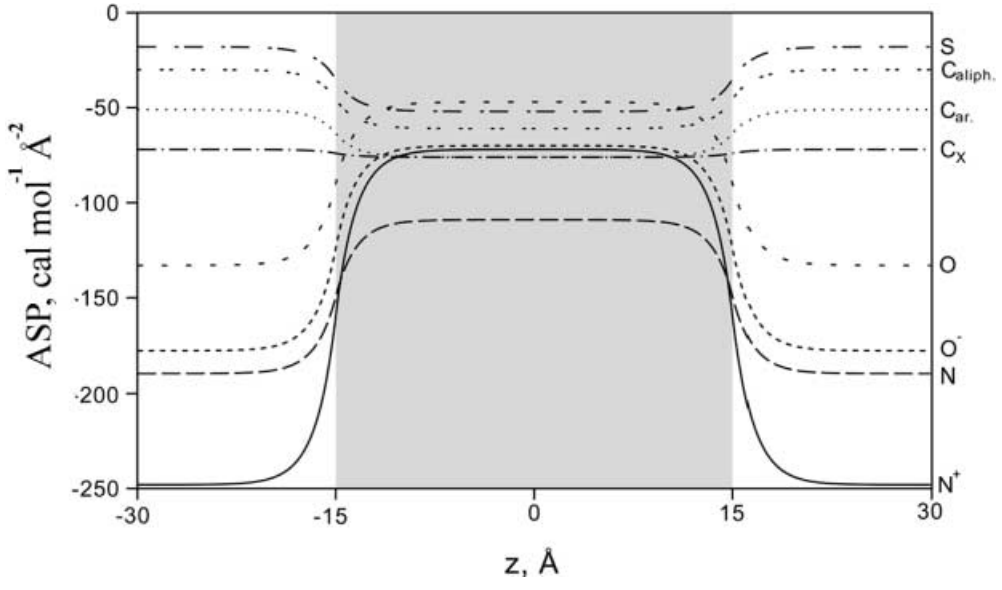

Fig. 1. Atomic solvation parameter (*ASP*) values versus coordinate z for different atom types used in the membrane model. The atom types are aliphatic carbon, C_{aliph}, aromatic carbon, C_{ar}, carbon atom bonded to any heteroatom (including those in aromatic rings), C_x, sulfur, S, uncharged and charged oxygen, O and O^-, respectively, and uncharged and charged nitrogen, N and N^+, respectively. A detailed description of the atom types is given in Ref. [5]. The nonpolar layer of the membrane is *hatched*

1. The properties of the system are constant in the membrane (xy) plane and vary along the z-axis, which is perpendicular to the membrane plane.
2. The membrane thickness (D) is constant.
3. The interaction of protein both with aqueous and membrane environments is given by Eq. (2), where ASP_i depends on the z coordinate of the atom.

Assuming that apart from the water–membrane interfaces, the values of the ASPs should correspond to those for bulk water and cyclohexane, respectively, we propose that

$$\mathrm{ASP}_i(z) = \begin{cases} \mathrm{ASP}_i^{\mathrm{mem}} - 0.5\left(\mathrm{ASP}_i^{\mathrm{mem}} - \mathrm{ASP}_i^{\mathrm{wat}}\right)\mathrm{e}^{(|z|-z_0)/\lambda}, & \text{if } |z| < z_0 \\ \mathrm{ASP}_i^{\mathrm{wat}} + 0.5\left(\mathrm{ASP}_i^{\mathrm{mem}} - \mathrm{ASP}_i^{\mathrm{wat}}\right)\mathrm{e}^{(|z|-z_0)/\lambda}, & \text{if } |z| \geq z_0 \end{cases}, \tag{2}$$

where $\mathrm{ASP}^{\mathrm{wat}}{}_i$ and $\mathrm{ASP}^{\mathrm{mem}}{}_i$ are the ASPs for atoms of type i determined either for aqueous (wat) or nonpolar (mem) environments, respectively, z_0 is the half-width of the membrane (i.e. planes of the bilayer are given by the equation $|z| = z_0$), and λ is a characteristic half-width of the water–membrane interface (usually 1.5 Å). We used $D = 2z_0 = 30$ Å. The model was incorporated into the FANTOM program [17].

The term $E_{\Delta V}$ was used only in simulations of GpA. It reflects the effect of the transbilayer voltage difference (ΔV):

$$E_{\Delta V} = \sum_{i=1}^{N} q_i V(z_i) = \sum_{i=1}^{N} \frac{q_i z_i \Delta V}{2z_0}, \tag{3}$$

where q_i and z_i are the partial charge and z-coordinate of atom i and z_0 is the half-width of the membrane.

2.2 Simulated objects

In the case of GpA (sequence SEPEITLIIFGVMAGVIGTILLISYGIRR), the starting structure was built in a random-coil conformation exposed to the polar phase of the system. A MC conformational search was performed with $\Delta V = 0$ mV. To explore during the simulation all possible orientations of the polypeptide chain with respect to the membrane (external, partially inserted, TM, and so on), a fragment of eight dummy residues was attached to its N terminus. The first atom of the N-terminal dummy residue was always placed in the center of the hydrophobic layer with coordinates (0,0,0). The number of dummy residues is determined by the membrane half-width and the protein size. These "virtual" residues do not contribute to the energy of the system. The starting structure of CTX corresponded to the major form found in aqueous solution by NMR [15]. It was taken from the Protein Data Bank (entry 1cb9). As for GpA, a fragment of 20 dummy residues was attached to its N terminus.

2.3 MC simulations

The polypeptide's conformational space was explored via an unrestrained MC search. All dihedral angles were sampled, except angles ω in "real" residues. The variation step of each dihedral was chosen randomly in the range $-180°\div180°$. Nonbonding interactions were truncated with a spherical cutoff of 30 Å. Long-range electrostatic interactions were damped using a distance-dependent dielectric permeability $\varepsilon = 4r$ (both in water and in the membrane). The choice of the dielectric screening model was discussed in detail elsewhere [11]. Before the MC simulation, the structure was subjected to 100 cycles of conjugate gradients minimization, and acceptance of the conformers was done according to the Metropolis criterion [18]. To cross the energy barriers between local minima, the adaptive-temperature schedule protocol [17] was employed. In the beginning of the MC search for CTX (first 5000 MC steps), a number of distance restraints obtained from NMR experiments in dodecylphosphocholine micelles was applied. The set of resulting structures was analyzed using the following parameters: total energy, secondary structure, degree of helicity (N_α), ASA, angles θ of helical segments with the z-axis, hydrogen bonding, z coordinate of the peptide's center of mass (z_{cm}). The following computational protocol was employed:

1. The initial MC run of 5000 steps with linearly decreasing temperature (initial $T = 2500$ K). The number of randomly chosen torsion angles in this run (N_{dih}) was 25. At each MC step the structure was minimized via 70 conjugate gradients iterations ($N_{min} = 80$).
2. Two MC runs of 5000 steps each with $N_{dih} = 15$ and 10, $N_{min} = 100$, and T linearly decreasing from 2000 K.
3. Two MC runs of 5000 steps each with $N_{dih} = 5$ and 1, $N_{min} = 100$, and T linearly decreasing from 1400 K.
4. Then the adaptive-temperature schedule protocol was employed during four consecutive runs (5000 iterations each) with $N_{dih} = 5$, 3, 2 and $N_{min} = 120$.
5. Several MC runs with $N_{dih} = 3,2,1$, $N_{min} = 50$, 120.

At each stage, the initial conformation was the lowest-energy one found at the previous stages. Stages 1–3 were used only for GpA. In total, 60000 and 35000 MC steps were performed for GpA and CTX, respectively. Other details of the simulations can be found in our previous works [5, 11, 19, 20].

To analyze the influence of the TM voltage, several additional MC runs were performed for GpA with $\Delta V = 500$ mV. In these cases, the starting structures were the lowest-energy ones (TM and hairpinlike (HP) states) obtained under the same conditions but with $\Delta V = 0$ mV, as described previously. A MC conformational search was carried out with $N_{dih} = 3$, 2 and $N_{min} = 20$, 50, 100.

3 Results and discussion

3.1 MC conformational search for GpA starting from a random-coil structure

A MC simulation ($\Delta V = 0$ mV) with a starting α-helical structure [19, 20] leads to low-energy states with a high degree of helicity (N_α). During the conformational search the system does not cross high-energy barriers, which separate the states with lower N_α. At the same time, information about them is important, because it can provide insight into processes such as peptide insertion into membranes, formation of secondary structurse, helix-forming properties of residues in membrane-mimetic media, and so forth. To examine such conformations of GpA, we carried out MC simulations starting from a random-coil structure.

The results obtained are presented in Figs. 2 and 3. Analysis of the conformers accumulated during the search permits the following conclusions to be drawn:

1. After the initial minimization the peptide is partially buried in the hydrophobic layer (Fig. 2A).
2. α-Helical fragments are preferentially observed near the C terminus (residues 19–22, Fig. 2B).
3. Upon decreasing the energy, additional helical segments appear in other parts of the sequence (Fig. 2D, E).
4. The conformers with one (Fig. 2C), two (Fig. 2D), or three (Fig. 2E) helical regions are trapped during the simulation.
5. The set of low-energy states reveals two structural motifs (Fig. 2F, G): the entire α helix in a TM orientation (TM-GpA) and a hairpin (HP)-like state with an α-helix break on residues Ala14 and/or Gly15 and both termini exposed on the same side of the hydrophobic layer (HP-GpA).
6. The energy difference (ΔE) between the lowest-energy TM and HP states is about 1 kcal/mol. The energy

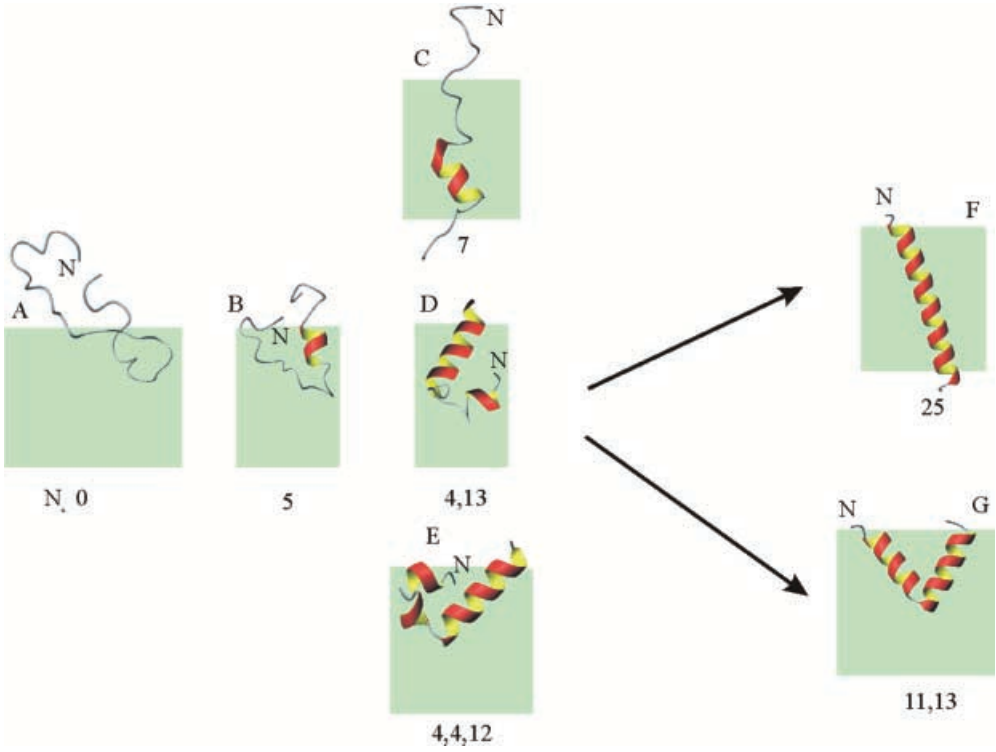

Fig. 2A–G. Results of Monte Carlo conformational search of glycophorin A (*GpA*) with a random starting structure. **A** Starting conformation after energy minimization. **B** Conformation with the first observed α-helical fragment. **C–E** Intermediate states of GpA. **F, G**: Low-energy transmembrane GpA (**F**) and hairpinlike GpA (**G**) states. The peptide is given in a ribbon presentation. The nonpolar layer of the membrane is *hatched*. The N terminus of GpA is marked with *N*. The numbers of residues in the α-helical segments are indicated

ranges characteristic for TM-GpA and HP-GpA are strongly overlapped, thus permitting the conclusion that the two states might be present in equilibrium.

The existence of two different states with close energies, namely TM- GpA and HP-GpA, calls for more detailed analysis of these structures because the available experimental data show that GpA spans a bilayer as a TM α helix [6]. Inspection of the lowest-energy conformers implies that

1. TM-GpA is stabilized by three additional "side chain–backbone" hydrogen bonds (Thr6:H1...O:-Glu2, Thr19:H1...O:Gly15, Ser24:H1...O:Ile20).
2. HP-GpA has two "side chain–backbone" hydrogen bonds (Thr6:H1...O:Pro3, Ser24:H1...O:Ile20) and one "side chain–side chain" hydrogen bond (Arg29:HH11...O:Glu4) connecting its extramembrane termini.
3. Residues Pro3-Arg28 are buried in the hydrophobic layer, while residues Ser1, Glu2, and Arg29 are exposed to the polar phase.

To summarize we can conclude that

1. All the low-energy conformers of GpA reveal high N_α values, and their hydrophobic regions are buried in nonpolar parts of the membrane.
2. In the voluminous set of the accumulated conformers, different elements of secondary structure (α helix, 3_{10} helix, several types of β turns, etc.) are detected. α-Helical segments are preferentially observed near the C terminus (residues 19–22). Upon decreasing the energy, some additional helical segments appear in other parts of the sequence (Fig. 3).
3. The most stable states have either TM (Fig. 2F, energy range from −301 to −257 kcal/mol) or HP (Fig. 2G, energy range from −300 to −279 kcal/mol) orientations.

We should note that, according to the experimental data [6], GpA reveals only TM orientation in the bilayer; no HP-like structures were detected. The possible reasons for the inconsistency of the MC results compared to the experimental observations might be as follows:

1. Experimental techniques used before 1985 (see references in Ref. [6]) failed to detect HP-like states of GpA. Thus, in recent studies of other peptides both states were found [21].
2. Limitations of the theoretical model – they are discussed later and in our previous works [11, 20]. One of them is caused by the symmetric nature of the membrane, although real membranes possess prominent asymmetry in the distribution of, for example, the electrostatic potential on their outer and cytoplasmic surfaces. We assume that a symmetric membrane might promote HP-like states of GpA. To check such an assumption, we later describe the results of simulations with the solvation model, which takes into account TM voltage and, hence, the asymmetry of the membrane.

3.2 Effect of TM voltage on the behavior of GpA

To analyze the effect of the TM potential on the behavior of GpA, two additional MC simulations with

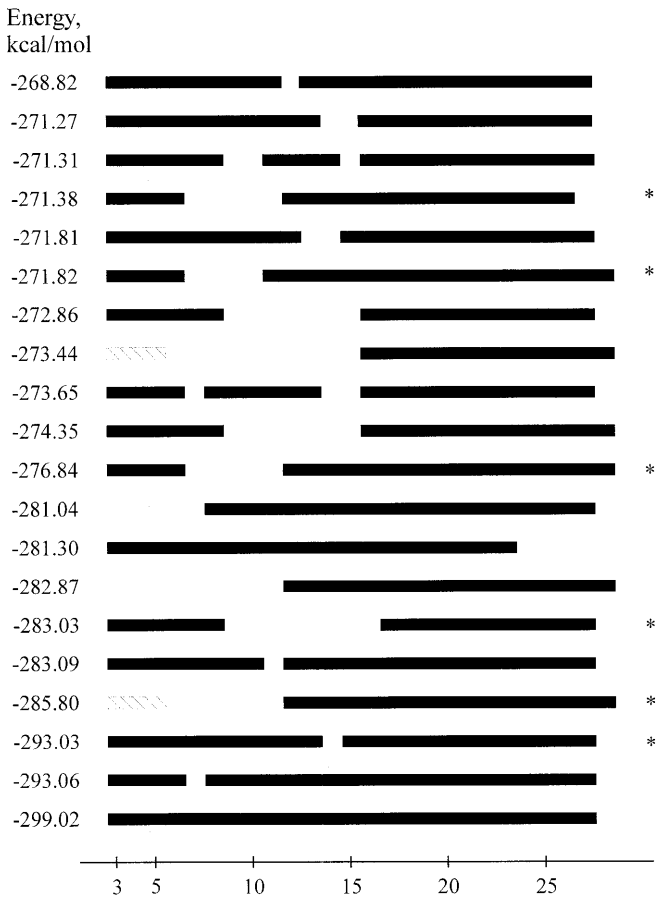

Fig. 3. Unique secondary structures of low-energy conformers of GpA obtained from the Monte Carlo simulation with a random starting structure. *Left*: the value of the energy for the most stable structure for each group of states. *Center*: α and 3_{10} helices are shown with *black* and *hatched rectangles*, respectively. *Right*: hairpinlike structures are indicated by *asterisks*

$\Delta V = 500$ mV were performed. The membrane thickness was taken as 30 Å. As follows from our previous data [19, 20], it corresponds well to the length of the hydrophobic part of GpA (so-called effect of "hydrophobic match–mismatch" [22]), thus stabilizing the TM-GpA state. The starting structures were the lowest-energy TM and HP states (Fig. 4A, B, respectively) found as described earlier with $\Delta V = 0$ mV. In this simulation, the membrane surfaces are not equivalent – the sides at $z = 15$ Å and $z = -15$ Å carry positive and negative charges, respectively. The peptide itself is characterized by a nonuniform distribution of charges along the sequence – it contains two negatively (Glu2, Glu4) and two positively (Arg28, Arg29) charged residues on the N and C termini, respectively. Therefore, one might expect that the amino terminus would be preferentially exposed to the polar layer with $z > 15$ Å, while the carboxyl one will tend to be on the opposite side of the hydrophobic medium.

The results of the simulations fully confirm such a proposal. As seen in Fig. 4, the lowest-energy states of GpA represent entire TM α helices ($N_\alpha = 26$, $\theta = 156°$). Its N terminus is accessible to water on the positively charged surface of the membrane (at $z > 15$ Å). As in the case of $\Delta V = 0$ mV, there are still two concurrent stable states – TM-GpA and HP-GpA. In addition, some other low-energy states have TM orientation but they are not all-helical and contain helix breaks on residues 17–18, 15–16, or 13–15. Their energies are about 1–13 kcal/mol higher than the most stable TM-GpA conformation. The principal finding is that the transbilayer voltage significantly (by about 15 kcal/mol) promotes TM orientation, whereas it has almost no effect on the HP state (Fig. 4). In a charged membrane, the energy difference between the most stable TM and HP states is 16 kcal/mol. We should note that the absolute value of the contribution of the voltage-dependent term ($E_{\Delta V}$) is negligibly small compared to $E_{\mathrm{ECEPP}/2}$ and E_{solv} (data not shown). The conclusion was made that mainly the altered balance between different energy terms causes the effect of ΔV.

Therefore, in simulations with the asymmetric membrane model the only set of energetically favorable states corresponds to the entire α helix, which spans the membrane. This agrees well with the experimentally observed structure of GpA in a membranelike environment [6]. Our current studies are being pursued to explore in more detail the stability of different states of GpA depending on the applied voltage. Also, this solvation model will be employed in simulations of voltage-dependent insertion of peptides into membranes.

3.3 Modeling of the binding of cytotoxin II to a membrane

The results of the MC simulation of CTX with the heterogeneous solvation model are presented in Fig. 5. In thzze beginning of the modeling procedure, the toxin molecule was placed in water, outside the hydrophobic layer of the membrane (Fig. 5-1). After the initial energy minimization and several thousands of MC steps, CTX was found to be adsorbed on the hydrophobic layer (Fig. 5-2, 5-3). In these states one, two, or all three toxin loops were in contact either with the membrane interface or with the nonpolar media. Subsequent unrestrained MC simulations resulted in a significant decrease in the energy of the system. One of the most energetically favorable states is shown in (Fig. 5-4).

Analysis of the accumulated low-energy conformers permits the following conclusions to be drawn:

1. The toxin molecule partially inserts into the membrane with the tips of its nonpolar loops – residues 6–10 (Leu-Val-Pro-Leu-Phe, loop I), 24–34 (Met-Phe-Met-Val-Ala-Ala-Pro-His-Val-Pro-Val, loop II), and 46–52 (Ser-Leu-Leu-Val-Lys-Tyr-Val, loop III).
2. Loops I and II strongly interact with the hydrophobic core, while loop III is buried with only three residues (47–49). In contrast, loop III reveals favorable interactions with the water–lipid interface. In the lowest-energy state, the energies of interaction of residues in loops I–III with the hydrophobic layer are −2.93, −10.27, and −1.76 kcal/mol, respectively.

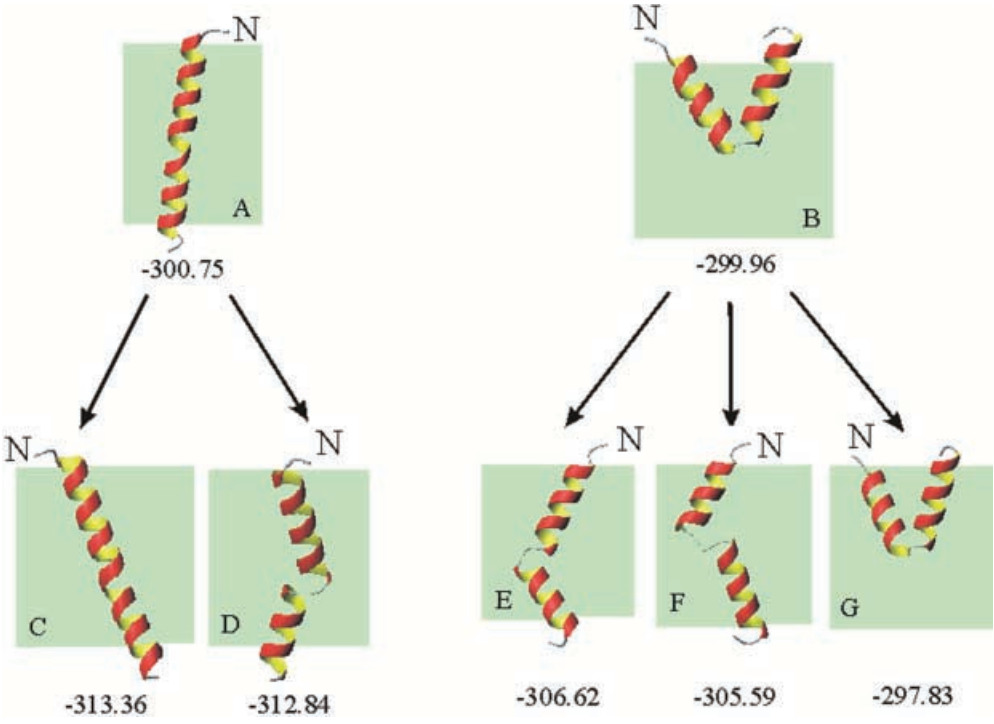

Fig. 4. Monte Carlo conformational search for GpA with applied transmembrane voltage. Starting structures (*top*) and representative groups of low-energy structures produced by the conformational search (*bottom*) for transmembrane (*left*) and hairpinlike (*right*) states. Corresponding values of total energy (in kcal/mol) are given for each state. Other details as in Fig. 2

Corresponding values for the protein-interface interaction are −2.44, −0.08, and −6.88 kcal/mol.

3. The overall spatial structure of the toxin is well retained in the membrane bound state compared to its water-soluble form – the root-mean-square deviation (rmsd) calculated over C_α atoms is 1.17 Å (rmsd for all heavy atoms is 2.55 Å). Minor differences are observed in the loop regions of CTX. This is not surprising because, like other three-finger toxins, CTX is extremely stable – it is cross-linked with four disulfide bridges and has a tightly packed β-structural core. It is interesting that all stable CTX conforma-

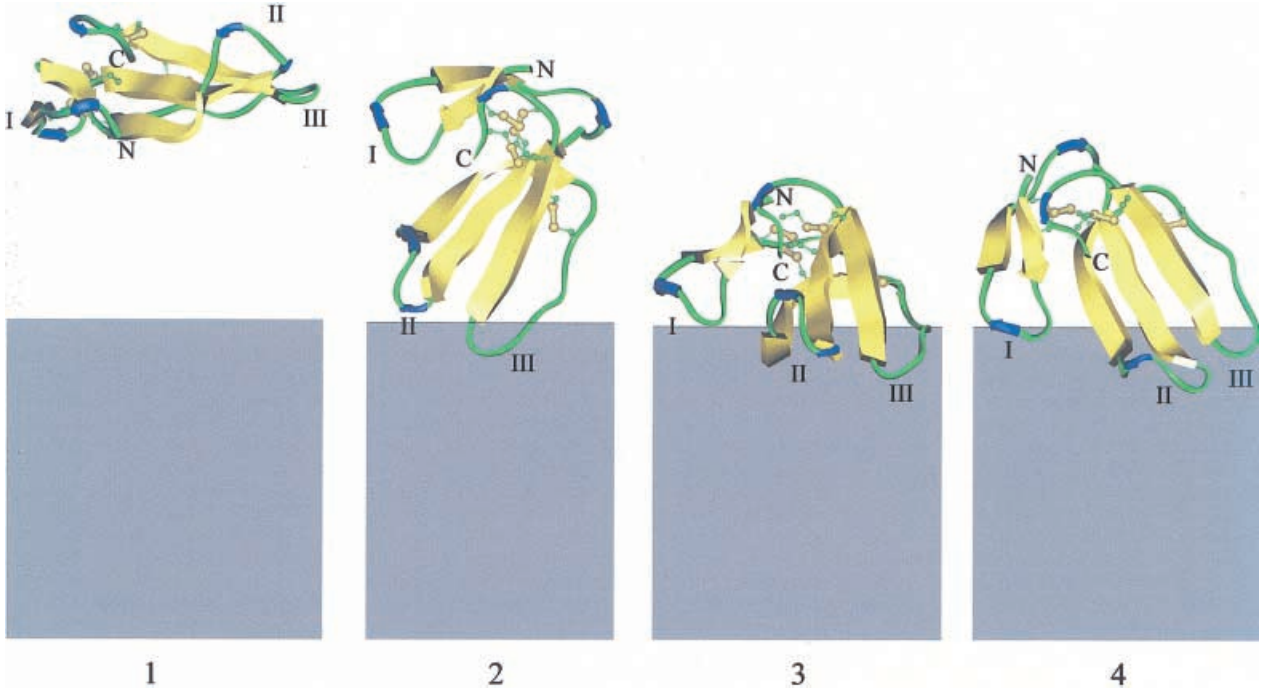

Fig. 5. Results of Monte Carlo simulation of cytotoxin II from *Naja naja oxiana* in membrane-mimetic media. *1* Starting structure. *2*, *3* Examples of the medium-energy states found *via* Monte Carlo conformational Search. *4* The lowest-energy state of cytotoxin II in the membrane. β sheets and β turns are displayed with *large* and *small arrows*, respectively. The toxin's loops are marked *I*, *II*, and *III*. Disulfide bridges are shown in a stick-and-ball representation. Other details as in Fig. 2

tions found in the membrane represent the major form observed in aqueous solution by means of NMR spectroscopy [15].

As in any study destined to search for stable states of a large biomolecular system, the challenge resides in the quality of sampling the conformational space. With regard to the method employed here, this question was addressed in our previous work [11] by inspection of all trial values of dihedrals and MC acceptance rates. It was found that the procedure ensures large conformational flexibility of the polypeptides studied and, therefore, allows exhaustive exploration of the potential-energy hypersurface. This is demonstrated by the fact that after $2–5 \times 10^4$ MC steps it was possible to get entire α helices of GpA (this work) and several other 20-residue peptides [11] starting from random-coil conformations. At the same time, we should mention that the problem of the effective search for a global minimum is still unresolved, and further improvements of the simulation protocols are indispensable.

To summarize, the computational results obtained for GpA and CTX demonstrate that, in spite of different methods of their binding to membranes, the low-energy states of the polypeptides found agree fairly well with known experimental data [13, 23]. This makes us confident that the approach may be successfully employed to study new biologically important membrane peptides and proteins. On the other hand, we should outline that the solvation model is rather approximate – a number of important characteristics of biological membranes, like heterogeneity of dielectric properties, density, chemical composition, microscopic nature of protein–lipid interactions, compressibility, and so forth, are not taken into consideration. Further work is required to refine the model and to delineate the main factors which govern protein behavior at water–membrane interfaces.

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