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Peptides in membranes: assessment of environmental effects via simulations using an implicit solvation model*

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Abstract. A recently developed implicit solvation model is applied to Monte Carlo simulations of peptides in bilayer-mimetic and polar environments. The model employs the formalism of atomic solvation parameters and reproduces experimental data. Solvent effects on the structure of the following peptides were studied: 20-residue poly-Leu and poly-Val, transmembrane helix A of bacteriorhodopsin, magainin2. It was shown that a membrane-like environment considerably promotes α-helix formation (all the peptides were found to be α-helical), while simulations in water reveal helix distortion. Consistency of the results with experimental data and further implications of the model are discussed.

Key words: Protein-membrane interactions – Molecular modeling – Monte Carlo method – Hydrophobic effect – Environment-dependent potential

1. Introduction

Understanding of the structure-function relationship for trans-bilayer peptides is an intriguing challenge in structural biology. These peptides participate in a variety of cell processes such as, ion transport, cell signaling, etc. It is now established that the peptide's conformation is greatly influenced by the membrane environment [1] and preferences of amino acid residues to form different types of secondary structure might differ in globular and membrane-bound states. At the same time, structural data on membrane systems is difficult to obtain experimentally, and only a few spatial models of membrane proteins are known [2]. Thus application of molecular modeling techniques with correct treatment of the effects of the membrane environment might be a promising alternative.

Various solvation models used in Monte Carlo (MC) and molecular dynamics simulations of membrane peptides and proteins are reviewed elsewhere [3]. The two most reliable ones are: (1) calculations with an explicit solvent, (2) off-lattice peptide models with implicit consideration of membrane effects. Although full atom representation 1 permits microscopic description of protein structure and dynamics, that method demands that a large number of parameters (solute and solvent degrees of freedom) are known with high precision [4]. Because the energetics of this system are determined by a subtle balance of strong intermolecular interactions, errors in calculation of free energy might occur. Also, it is CPU-time consuming. Off-lattice models [5] employ C_α representation of the peptide chain having helical propensities and an effective membrane-mimetic potential. Although they give good predictions both for the state of association and the orientation of the peptide relative to the membrane, these models are too simple and lack details important for peptide-lipid interactions. For example, the hydrophobic nature of residues is not taken into account and is replaced by a chain of C_α atoms. Also, the peptides are forced to adopt a helical conformation and, therefore, differences in secondary structure preferences are not explored.

Recently [3], we proposed an implicit solvation model based upon the formalism of atomic solvation parameters (ASP) and imitating effects of different environments. The approach combines more detailed protein presentation than that in off-lattice models, and is rather more computationally effective than explicit solvent description. In this study we present applications of the method to MC simulations of 20-residue poly-Leu and poly-Val, transmembrane (TM) helix A of bacteriorhodopsin (BRh-A) and magainin2. The first part of the paper deals with testing of the membrane-promoting effects on α-helix formation for homo-polypeptides. Then we describe the conformational behavior of BRh-A and magainin2 in different

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environments and compare the results with experimental data.

2 Method of calculation

The coordinates of BRh-A (residues 10–29) were taken from the Brookhaven Protein Data Bank entry 1BRD [6]. Magainin2 from *Xenopus laevis* (sequence: GIGKFLHSAGKFGKAFVGEIMKS) was constructed in α-helical conformation. Initial structures of 20-residue poly-L-Leu and poly-L-Val were built in random coil conformations using the FANTOM program [7]. The peptides were taken with neutral N- and C-terminal groups: N-Ac/N-Met for homo-polypeptides; NH_2/COOH for BRh-A and magainin2. The potential energy function was $E_{\text{total}} = E_{\text{ECEPP/2}} + E_{\text{solv}}$. The term $E_{\text{ECEPP/2}}$ includes van der Waals, torsion, electrostatic and H-bonding contributions [8]; $E_{\text{solv.}}$ is a solvation energy, $E_{\text{solv.}} = \sum_{i=1}^{N} \Delta\sigma_i \text{ASA}_i$, where ASA_i and $\Delta\sigma_i$ are the accessible surface area and atomic solvation parameter of atom i; N is the number of atoms. The ω angles were fixed, and a spherical cutoff was set to 30 Å for nonbonding interactions. Because the pseudo-energy term $E_{\text{solv.}}$ was simply added to the ECEPP/2 (vacuum) potential, and $E_{\text{ECEPP/2}}$ was not used to derive ASPs from experimental data, employment of such a hybrid force field might lead to double accounting of, e.g., electrostatic interactions appearing in $E_{\text{ECEPP/2}}$ and implicitly included in $E_{\text{solv.}}$. To avoid this (at least partly), long-range electrostatic interactions were dumped by using distance-dependent dielectric permeability $\varepsilon = 4 \times r$, although the short-range ones contributing to the H-bonding term were explicitly included.

The conformational space of the peptides was explored in nonrestrained MC simulations in torsion angle space in vacuo and with ASPs for gas-water and gas-cyclohexane transfer which imitate water and the hydrophobic core of a membrane, respectively [3]. For BRh-A and magainin2 the simulation length was 2000 MC cycles. Identical starting conformations were used in simulations of the same peptide with different ASPs. For poly-Leu and poly-Val the following protocol was employed:

1. Initial random structures were subjected to 5700 steps of MC conformational search with linearly decreasing temperature (initial $T = 2000$ K). At each MC step, ten randomly selected dihedral angles were sampled and the current structure was minimized via 100 conjugate gradient iterations.
2. Then the adaptive-temperature schedule protocol [7] was employed during 2000 iterations by sampling five randomly selected dihedrals followed by 150 minimization steps. The initial conformation was the lowest-energy structure found in step 1.
3. Finally, a protocol similar to that in step 2 but with one dihedral angle sampled was applied during 1000 MC steps. Other details of the simulations could be found in Ref. 3. Hydrophobic properties of magainin2 were calculated using the molecular hydrophobicity potential (MHP) approach as described elsewhere [9]. Ribbon diagrams of the molecules were pictured with the MOLMOL program [10].

3 Results and discussion

3.1 Poly-Leu and poly-Val

It is well known that helix formation is significantly promoted in bilayers [1]. Thus, C_β-branching residues such as Val and Ile are often found in TM α-helices, but reveal helix-destabilizing properties in water [11]. Therefore, solvent models imitating a membrane should reproduce helix-forming propensities for certain residues that are larger than those in water. To address this problem, we employed our sets of ASPs to study conformational properties of Val and Leu in vacuo and with ASPs which mimic nonpolar solvents and water. Leu was chosen as a reference residue because it has high helix-forming propensities in water and in the bilayer [12]. The peptides in initial random conformations were subjected to a nonrestrained conformational search, and the energy-minimized structures accepted by the Metropolis criterion [13] were selected for subsequent analysis. Only one type of secondary structure, α-helix, was found. The number of residues in α-helical structure versus the total energy of the system is shown in Fig. 1. Each point on these plots corresponds to a local minimum on the potential energy hypersurface characteristic for a given peptide in a particular solvent. The following inferences can be made:

1. In nonpolar media the lowest-energy conformations are all-helical and can span the bilayer.
2. In water a population of conformers with only one helix turn (4 residues) is found.
3. In vacuum numerous structures with significant helical content are found, although the lowest-energy ones do not reveal the maximal degree of helicity.
4. The energy gaps between the lowest-energy conformers with and without α-helices are higher in a nonpolar solvent than in vacuum: 78.05, 51.07 kcal/mol and 33.62, 6.77 kcal/mol for poly-Leu and poly-Val, respectively. Therefore, nonpolar solvents and, to a lesser degree, vacuum, promote α-helix formation, although the conformational landscapes are different. That is why one should take care when simulating TM segments in vacuo. In water, stable α-helices were observed only for poly-Leu, although it contained only 4 residues. This is consistent with the fact that water generally destabilizes α-helices due to competition with formation of H-bonds within the peptide backbone [14].

Membrane-promoting helix formation of Val was recently demonstrated by Circular Dichroism spectroscopy [1]. It was shown that although its C_β-branched side chain may sterically interfere with the carbonyl oxygen in the preceding turn of the helix and hence, destabilize it [15], in a nonpolar solvent this effect may well be balanced by favorable interactions of the hydrophobic side chain with solvent. Thus, in micelles and vesicles Val-containing peptides adopt conformations with high helical content. The results of our simulations agree with these experimental observations, and, moreover, provide additional insight into details of the energetic landscape of the peptides in different environments.

3.2 Transmembrane segment A of bacteriorhodopsin

BRh is an integral membrane protein which pumps protons across the cell membrane in response to light absorption. Electron microscopic study of the whole protein reveals that its membrane has an α-helical conformation [16], and NMR data show that BRh-A is helical in its monomeric state in micelles [17]. This makes BRh-A a suitable model to test the solvation model. The results of MC simulations of BRh-A in

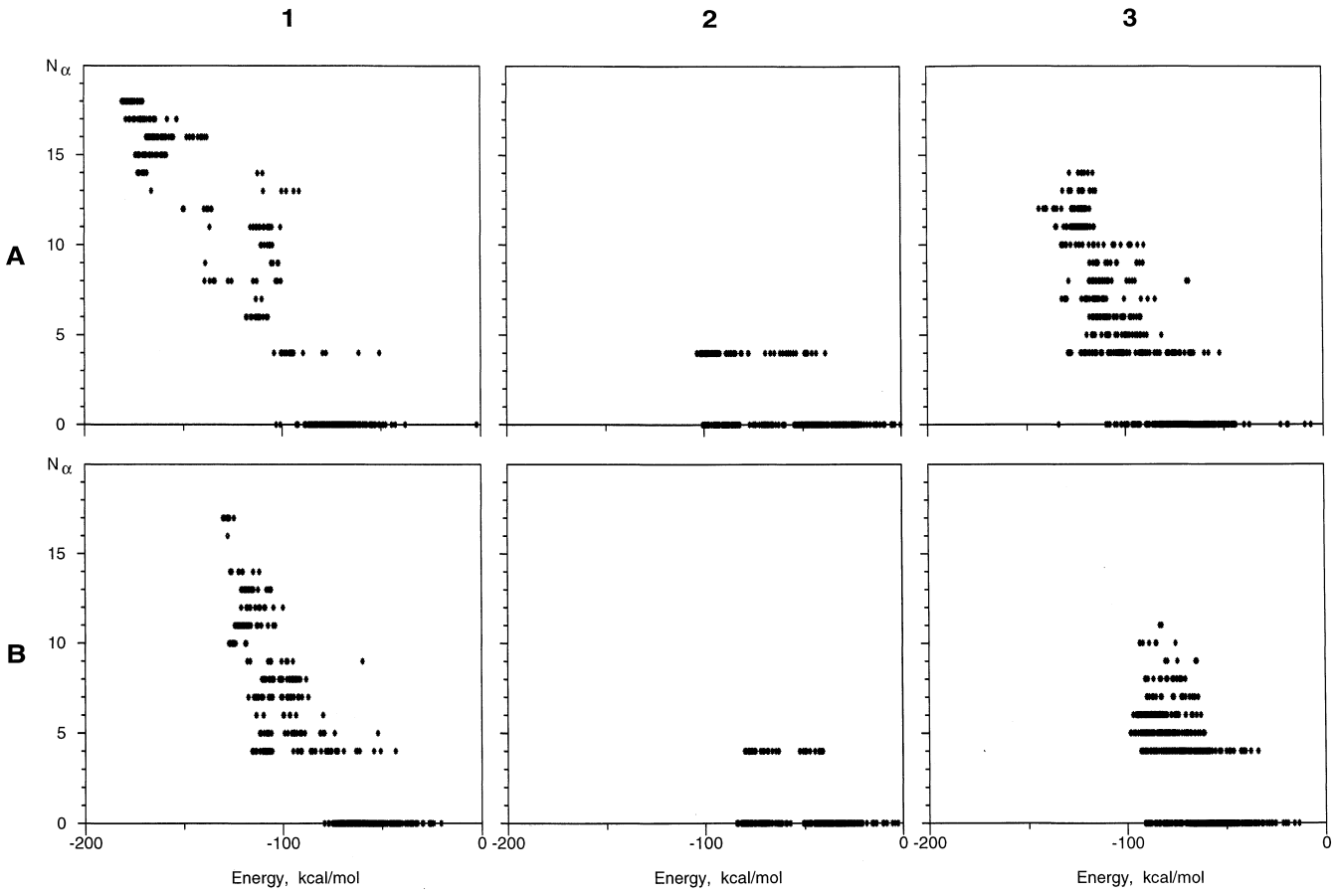

Fig. 1. α-Helical content versus total energy for accepted conformers of poly-Leu (**A**) and poly-Val (**B**) found by MC conformational search in a nonpolar solvent (**1**), water (**2**), vacuum (**3**)

vacuo, nonpolar solvent, and water are illustrated in Figs. 2A–C and 3: the lowest-energy conformers are given in Fig. 2, whereas Fig. 3 shows the dihedral angles φ, ψ, and χ^1 for the accepted conformers. The following conclusions can be made:

Hydrophobic solvent: (1) peptide retains α-helical conformation; (2) side chains of residues are more flexible than in water, but less flexible than in vacuum; (3) accepted conformers do not contain residues in nonhelical conformation; (4) as shown earlier [3], resulting χ^1 rotamers agree well with those obtained in an NMR study of BRh-A in sodium dodecyl sulfate (SDS) micelles.

Polar solvent and vacuum: (1) the middle part of the helix is destabilized; there is tight packing with significant decrease of accessible surface area; (2) there is a rather high degree of the backbone flexibility as compared with a hydrophobic solvent (Fig. 3); (3) in water, the following residues were found in non-helical conformation: Leu13, Gly16, Ala18, Gly21, and Leu22; (4) only residues 14–20, 24, 26, 27 form an α-helix in vacuum, whereas the others are disordered.

A question arises: how can simulations with ASPs representing a bulk solvent be used to mimic the heterogeneity of a membrane? We note that the objective of

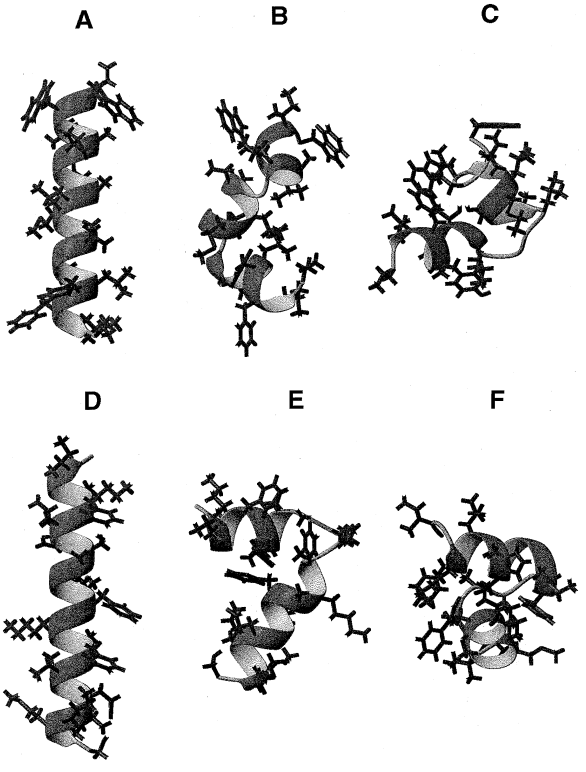

Fig. 2A–E. Ribbon representation of the lowest-energy conformers obtained via MC simulations. BRh-A in nonpolar solvent (**A**), water (**B**), vacuum (**C**), **D–F** The same for magainin2

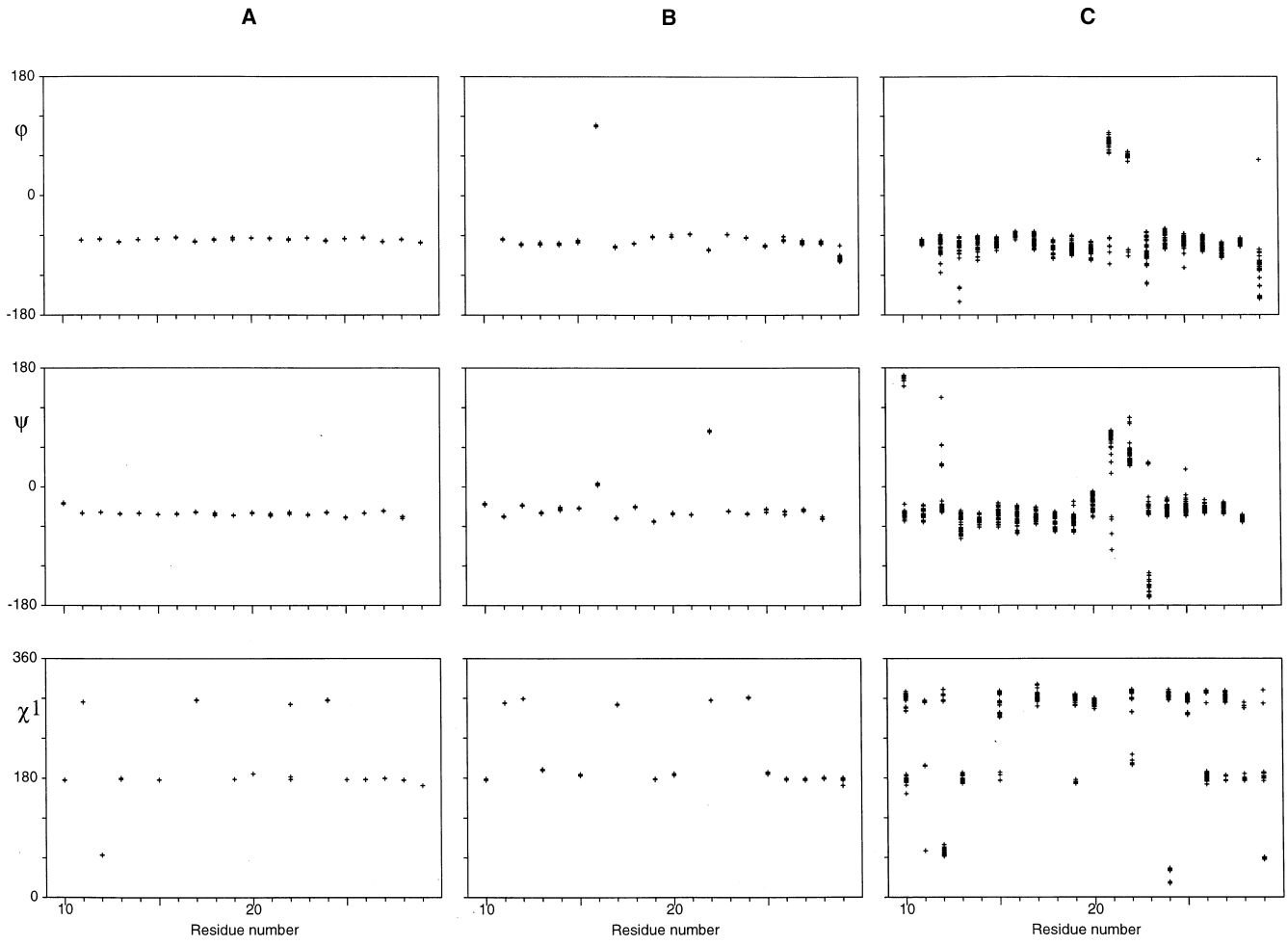

Fig. 3. Dihedral angles φ, ψ, χ^1 (plotted vs residue number) in the conformers of BRh-A obtained in MC simulations in nonpolar solvent (**A**), water (**B**), vacuum (**C**)

this work is to check the influence of different ASPs on conformational, H-bonding, etc. properties of peptides which are assumed to traverse a bilayer (except model systems like homo-polypeptides). In this case most of the peptide is immersed in a nonpolar core of the membrane and could be properly described by the parameters for gas-cyclohexane transfer (ASP_{gc}). For BRh-A the lowest-energy conformations obtained in simulations agree well with those derived by NMR in SDS micelles [17]. Small deviations are observed only for one or two terminal residues which are supposed to be outside the hydrophobic core of the bilayer (data not shown).

3.3 Magainin2

Magainin2 is a 23-residue antimicrobial peptide from the *Xenopus* skin which binds to the cell membrane [18]. MC simulations of magainin2 in different solvents and in vacuo show similar tendencies to BRh-A. Thus, the lowest-energy conformers in nonpolar media are totally α-helical (Fig. 2D), while those in water and in vacuum are disordered in the middle part and near the termini (Fig. 2E, F). It is interesting to assess the spatial hydrophobic properties of the lowest-energy all-helical conformer obtained in hydrophobic media. Figure 4 shows the distribution of hydrophobicity on the peptide surface as two-dimensional and one-dimensional molecular hydrophobicity potential (2D-MHP, 1D-MHP) plots. (Only hydrophobic regions with high values of MHP are indicated on 2D-MHP map). It is seen that the peptide has pronounced amphiphilic character with its nonpolar side formed by Ile2, 20, Phe5, 12, 16, Leu6, Ala9, Val17, and Met21 (rotation angle around the helix axis lies in the range 15°–180°), while the rest of the surface is polar (range 180°–360°). Taken together, these results suggest that magainin2 forms an α-helix in the bilayer. Most probably, due to its amphiphilic properties it does not span the membrane, but stays adsorbed on the lipid-water interface. In contrast, it does not form a stable α-helix in water. This agrees well with the results of NMR studies in detergent micelles and aqueous solutions ([5] and references therein).

4 Conclusions

The proposed solvation model, being employed in MC simulations of peptides, correctly accounts for the effects of a membrane-like environment and water. ASPs imitating a hydrophobic core of the bilayer significantly

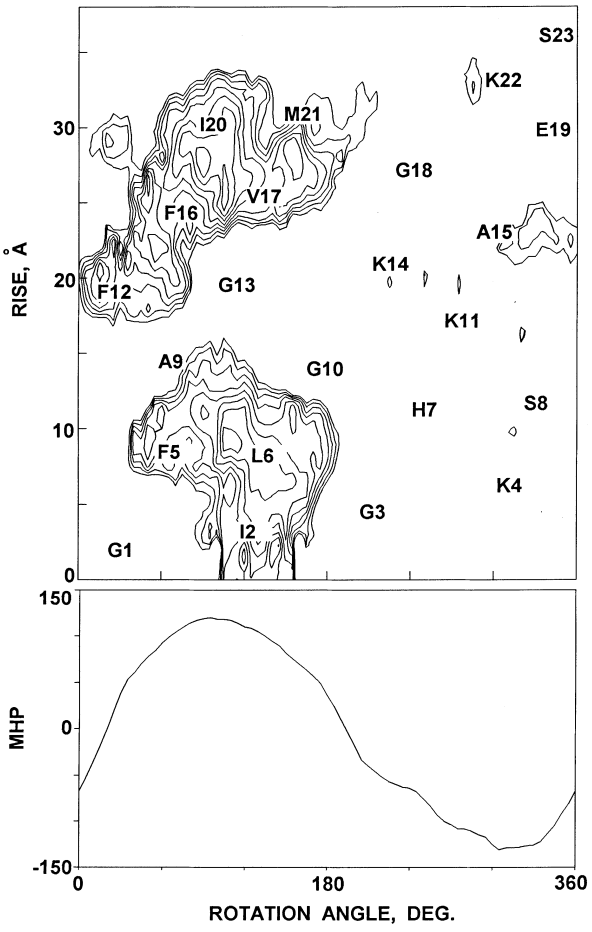

Fig. 4. Hydrophobic properties of the lowest-energy conformer of magainin2 found in MC simulation in a nonpolar solvent. (**Top**) Two-dimensional isopotential map of the molecular hydrophobicity potential (*MHP*) on the peptide surface. The value on the horizontal axis is the rotation angle about the helix axis; the vertical axis shows the distance along the helix axis. Only areas with MHP > 0.1 are shown. Contour intervals are 0.015. The residues are indicated as: I = Ile, F = Phe, L = Leu, A = Ala, V = Val, M = Met, G = Gly, K = Lys, E = Glu, S = Ser, H = His (**Bottom**) Angular distribution of MHP on the surface. MHP is summed inside the sectors 90° width

promote α-helix formation. Thus, starting from random coil structures, a conformational search in nonpolar media leads to all-helical lowest-energy conformers for poly-Leu and poly-Val. Also, BRh-A and magainin2 retain well their initial α-helical structures. Comparison with experimental data shows reasonable overall agreement. In contrast, ASPs for water induce helix distortion. Vacuum simulations reveal only partial helix formation and, therefore, do not reproduce correctly the structural and energetic properties of peptides in membranes. Current studies are pursuing development of a three-phase model of membranes based on combined usage of ASPs for water and hydrocarbon. The problems of peptide partitioning on the water-bilayer interface and membrane insertion will be addressed using this model.

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References

1. Deber CM, Goto NK (1996) Nature Struct Biol 3:815
2. Walker JE, Saraste M (1996) Curr Opin Struct Biol 6:457
3. Nolde DE, Vergoten G, Arseniev AS, Efremov RG (1997) J Biomol Struct Dyn 15:1
4. Edholm O, Jahning F (1988) Biophys Chem 30:279
5. Milik M, Skolnick J (1993) Proteins 15:10
6. Bernstein FC, Koetzle TF, Williams GJB, Meyer EF, Brice MD, Rogers JR, Kennard O, Shimanouchi T, Tasumi M (1977) J Mol Biol 112:535
7. von Freyberg B, Braun W (1991) J Comp Chem 12:1065
8. Némethy G, Pottle MS, Sheraga HA (1983) J Phys Chem 87:1883
9. Efremov RG, Vergoten G (1995) J Phys Chem 99:10658
10. Koradi R, Billeter M, Wüthrich K (1996) J Mol Graphics 14:51
11. Padmanabhan S, Marqusee S, Ridgeway T, Laue TM, Baldwin RL (1990) Nature 344:268
12. Blaber M, Zhang X-J, Matthews BW (1993) Science 260:1637
13. Metropolis N, Rosenbluth AW, Teller AH, Teller E (1953) J Chem Phys 21:1087
14. Tirado-Rives J, Jorgensen WL (1991) Biochemistry 30:3864
15. O'Neil KT, DeGrado WF (1990) Science 250:646
16. Grigorieff N, Ceska TA, Downing KH, Baldwin JM, Henderson R (1996) J Mol Biol 259:393
17. Pervushin KV, Arseniev AS (1992) FEBS Lett 308:190
18. Zasloff M (1987) Proc Natl Acad Sci USA 84:5449