A Solvent Model for Simulations of Peptides in Bilayers. II. Membrane-Spanning α-Helices

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ABSTRACT We describe application of the implicit solvation model (see the first paper of this series), to Monte Carlo simulations of several peptides in bilayer- and water-mimetic environments, and in vacuum. The membrane-bound peptides chosen were transmembrane segments A and B of bacteriorhodopsin, the hydrophobic segment of surfactant lipoprotein, and magainin2. Their conformations in membrane-like media are known from the experiments. Also, molecular dynamics study of surfactant lipoprotein with different explicit solvents has been reported (Kovacs, H., A. E. Mark, J. Johansson, and W. F. van Gunsteren. 1995. *J. Mol. Biol.* 247:808–822). The principal goal of this work is to compare the results obtained in the framework of our solvation model with available experimental and computational data. The findings could be summarized as follows: 1) structural and energetic properties of studied molecules strongly depend on the solvent; membrane-mimetic media significantly promote formation of α-helices capable of traversing the bilayer, whereas a polar environment destabilizes α-helical conformation via reduction of solvent-exposed surface area and packing; 2) the structures calculated in a membrane-like environment agree with the experimental ones; 3) noticeable differences in conformation of surfactant lipoprotein assessed via Monte Carlo simulation with implicit solvent (this work) and molecular dynamics in explicit solvent were observed; 4) in vacuo simulations do not correctly reproduce protein-membrane interactions, and hence should be avoided in modeling membrane proteins.

INTRODUCTION

Understanding the structure-function relationship for transbilayer peptides constituting either membrane-bound domains in proteins or functioning autonomously represents an intriguing challenge in the field of structural biology. It is now established that peptide conformation is greatly influenced by the environment (e.g., Kelly, 1998), and a number of chameleon-like molecules have been discovered which, being placed in different surroundings, are able to change their secondary and/or tertiary structure (Mihara and Takahashi, 1997). Indeed, preferences of amino acid residues to form or destabilize different types of secondary structure, which were characterized by many researchers in globular proteins (e.g., Fasman, 1989), may differ from those in the membrane-bound state (Deber and Goto, 1996). Unlike water-soluble proteins, structural properties of membrane-embedded systems are studied less because of limitations of current experimental methods in working with such complex systems. In this situation, important insights into the problem of peptide adsorption on the bilayer, membrane insertion, and stability in the membrane-bound state could be achieved through computer simulations. At the same time, successful application of these techniques requires correct treatment of solvent effects.

Various solvation models used in Monte Carlo (MC) and molecular dynamics (MD) studies of membrane peptides and proteins, along with their advantages and shortcomings, were discussed in the accompanying article. Among them, the models with implicit consideration of membrane effects 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 continuous medium whose properties vary along the membrane thickness, and membrane insertion is simulated using either MC or MD methods. Off-lattice (Milik and Skolnick, 1993) or full-atom (Ducarme et al., 1998) representations of the peptide molecules are used. Comparison of the results obtained in the framework of such models with experimental data shows that the calculations give good predictions both for the association state and peptide's orientation relative to the membrane surface. Moreover, implicit solvent models provide a number of insights into the mechanism of the peptides' insertion into membranes. At the same time, during the simulations the peptide's conformation was often fixed to the α-helix, and no changes of the structure were allowed. Hence, the problems related to conformational rearrangements, like formation or destabilization of the secondary structure induced by the environment, could not be addressed by these techniques.

Based upon formalism of atomic solvation parameters (ASP), in the first article of this series we proposed an implicit solvation model that mimics effects of membrane environment in simulations of peptides. In this model, an additional (solvation) term was incorporated into the ECEPP/2 potential and the resulting force field was tested in

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MC simulations of a number of small peptides and 20-residue homopolypeptides made of Leu, Val, Ile, and Gly. The main objective of this study is to inspect the solvation model, along with the method of exploring conformational space, by applying them to MC simulations of rather complex systems: membrane-bound peptides. Apart from validation of the approach, such computer experiments are able to provide new interesting structural and functional information about the peptides in a particular environment, which is difficult to access with the experimental techniques. An important feature of the model is total conformational variability of the molecules under study, which permits investigation of solvent effects on conformational properties. The first part of the paper deals with modeling of environmental effects for transmembrane (TM) α-helices A and B of bacteriorhodopsin (BRh), whose structure in membrane-mimetic media is known from the experiment. Then we describe conformational behavior of magainin2 and the hydrophobic segment of surfactant lipoprotein (SP-C), which also were extensively studied in the experiments, although the atomic-scale structures are not yet available. The calculated properties are compared with the experimental data and the results of MD simulations in explicit solvents reported by Kovacs et al. (1995).

METHOD OF CALCULATION

The coordinates of TM segments A (BRh-A, residues 10–29) and B (BRh-B, 34–65) of BRh were taken in the Brookhaven Protein Data Bank, PDB (Bernstein et al., 1977), entry 1BRD. In the initial structures, the following residues were in α-helical conformation: 11–28 in BRh-A and 34–65 in BRh-B. Before the calculations, hydrogen atoms were added to the PDB models. TM segments of human SP-C (residues 6–35) and magainin2 from *Xenopus laevis* (residues 83–105) were built as α-helices. This was done using the FANTOM program (von Freyberg and Braun, 1991). All the peptides were taken with neutral N- and C-termini.

The conformational space of peptides was explored in nonrestrained, variable-temperature MC simulations in torsion angle space in vacuo and with ASPs imitating water and hydrophobic core of a membrane, as described in the first article of this series. Details of MC simulations and analysis of the results can be also found there. The simulation length was 2000 MC cycles. After each MC iteration, the structures were subjected to conjugate gradient energy minimization. SP-C was also simulated by the MC method during 14,000 steps with ASP_{gc} (gas-cyclohexane) and ASP_{gw} (gas-water) at constant temperature (T = 300 K) and without energy minimization. In this last case the starting conformation was that found in the result of variable-temperature MC simulation in cyclohexane. Identical starting conformations were used in simulations of the same peptide in different environments. Accessible surface area and secondary structure were assessed using the DSSP program (Kabsch and Sander, 1983). Ribbon drawings of molecules were produced with the MOLMOL program (Koradi et al., 1996).

RESULTS AND DISCUSSION

To date, spatial structures of a number of peptides in membrane-mimetic environments (micelles, mixtures of organic solvents) have been established in the experiment. This provides a good basis for testing and refinement of theoretical solvation models suitable for simulations of effects of the bilayer on structural properties of peptides and proteins. To check ASPs, we chose the following objects: TM helices BRh-A and BRh-B, SP-C, and magainin2.

Transmembrane segment A of bacteriorhodopsin

BRh is an integral membrane protein that pumps protons across the cell membrane in response to light absorption. Electron cryomicroscopic (Grigorieff et al., 1996) data obtained for the whole protein as well as NMR studies of its TM components (e.g., Pervushin and Arseniev, 1992) reveal α-helical conformation of the membrane moiety. Moreover, some individual membrane-spanning peptides (e.g., BRh-A, BRh-B) retain their helicity well being in the monomeric state in micelles or in chloroform/methanol solvent (Pervushin and Arseniev, 1992; Lomize et al., 1992). Here we explore conformational space of α-helices BRh-A and BRh-B in different environments through variable-temperature MC conformational search.

Resulting distribution of the backbone (φ, ψ) and side chain (χ^1) torsion angles in BRh-A conformations in cyclohexane, water, and vacuum, are shown in Fig. 1. Only the low energy conformers (50% of all accepted structures) were taken for the analysis. Ribbon representation of the lowest-energy conformers is shown in Fig. 2. As seen in Fig. 1 *A*, in simulations with ASPs imitating nonpolar environment (set ASP_{gc}), the initial α-helical geometry is stable during conformational search: the angles φ and ψ retain the values characteristic for α-helix. None of the accepted conformers contains residues in nonhelical conformation. The lowest-energy structure reveals two side chain/backbone hydrogen bonds (H-bonds): Leu-13:O$\cdots$H$^{\gamma 1}$:Thr-17; Met-20:O$\cdots$H$^{\gamma 1}$:Thr-24. It is important to note that these H-bonds are observed experimentally for BRh-A incorporated into micelles (Pervushin and Arseniev, 1992).

As seen in Fig. 1 *B*, simulations with ASPs imitating polar solvent (ASP_{gw}) reveal distortion of the α-helical conformation for residues Gly-16 and Leu-22: they are assigned to H-bonded turn ("T") and five-residue bend ("S") types of secondary structure. [Here after, identifiers of the secondary structure are given in the notation of the DSSP algorithm (Kabsch and Sander, 1983).] Interestingly, the helix unfolding was always observed near the middle of the peptide, whereas the termini were relatively stable. Simulation in vacuum (Fig. 1 *C*) reveals that only residues Ala-14–Met-20, Thr-24, Tyr-26, and Phe-27 still retain α-helical conformation, whereas the others can adopt the different ones (in the lowest-energy structure Ile-11, Trp-12, Leu-13, Leu-19, and Met-20 are assigned to "T" conformation, while Leu-22 is assigned "S"). In vacuum the helix is significantly distorted at the ends and in the middle, and two helical fragments (Leu-15–Met-20 and Gly-23–Leu-28) are tightly packed (Fig. 2 *C*) resulting in considerable decreasing of ASA. Thus, values of total ASA for the lowest-energy conformers in cyclohexane, water, and vacuum are 2250, 2050, and 1770 Å^2, respectively. The structure in vacuum has two side chain/backbone (Leu-13:O$\cdots$H$^{\gamma 1}$:

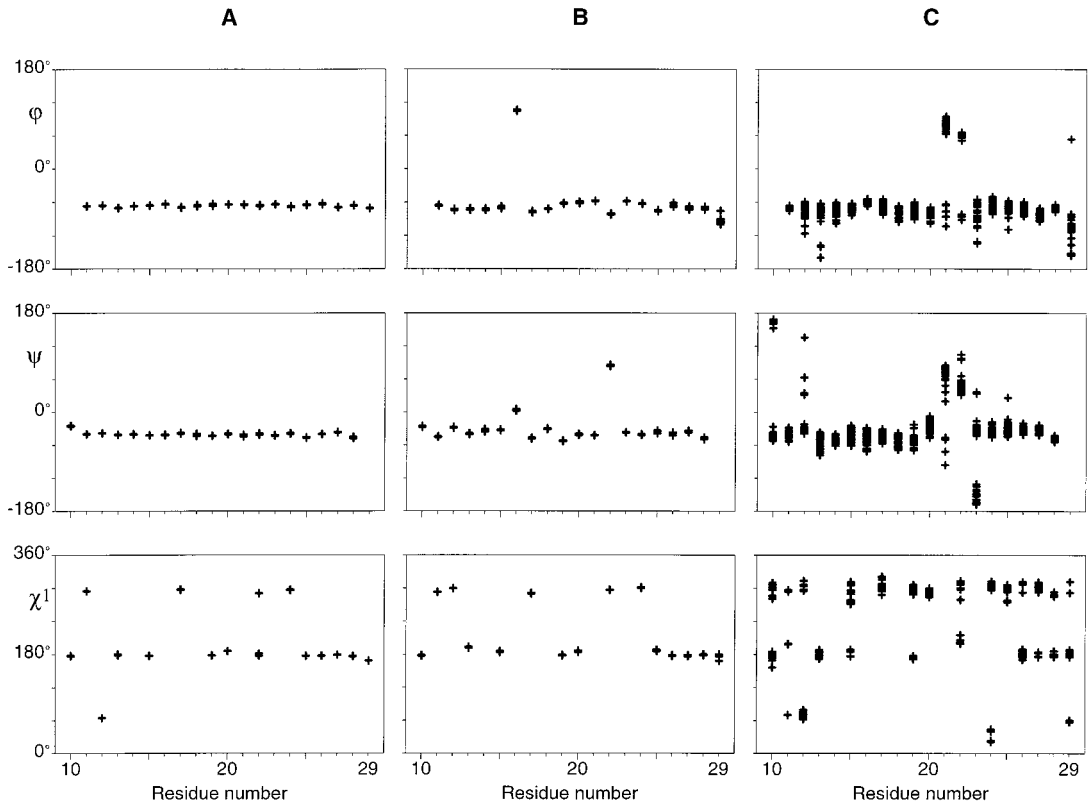

FIGURE 1 Dihedral angle distribution during Monte Carlo simulations of transmembrane segment BRh-A at variable temperature with the following sets of atomic solvation parameters (ASP): (*A*) ASP_{gc}; (*B*) ASP_{gw}; (*C*) in vacuum. φ, ψ, and χ^1 angles in 50% of low-energy accepted conformations are plotted versus the residue number.

Thr-17; Trp-12:O· · ·$H^{\gamma 1}$:Thr-24) and one side chain/side chain (Thr-17:$H^{\gamma 1}$· · ·$O^{\gamma 1}$:Thr-24) H-bonds.

Inspection of energies of the conformers accepted during the search in different environments (Table 1) shows that the all-helical structure in nonpolar solvent is present within a wide range of energies: $\Delta E = 28.9$ kcal/mol, where ΔE is the difference between maximal and minimal energies in the set of conformers, although the energy distribution is centered near the lowest-energy value (−196.8 kcal/mol): the mean and standard deviation are −193.4 and 5.8 kcal/mol, respectively. In polar media, the all-helical state is observed only within a narrow interval of energies ($\Delta E = 5.4$ kcal/mol). Other states with one and two residues in nonhelical conformation have values of ΔE equal to 4.0 and 12.8 kcal/mol, respectively, and the last one corresponds to the lowest energy minimum. The α-helix is rather less favorable in vacuum: a broad spectrum of conformers with a helical content from 18 to 4 residues was obtained. Although the lowest-energy state ($E = -156.9$ kcal/mol) reveals 11 residues in α-helix (Fig. 2 *C*), several close states with minimal energies −156.4, −156.3, and −155.2 kcal/mol have 5, 10, and 12 residues in α-helix, respectively. Moreover, energy intervals of these states overlap between each other, and therefore the peptide could adopt multiple alternative conformations separated by low energy barriers.

As seen in Table 1, in all the cases the van der Waals term dominates the others, whereas relative values of different energy contributions depend on the solvent used. The ratio between van der Waals, solvation, and H-bonding terms in cyclohexane is similar for all the conformers. On the contrary, in water and vacuum the structure is determined by a balance of the terms, thus leading to multiple states with close total energies. For example, in water distortion of an initial all-helical structure (with $N_\alpha = 18$, where N_α is a number of residues in α-helix) is governed by decreasing E_{VDW} and E_{solv}, which is energetically more favorable than the accompanying breaking of C═O. . .H—N H-bonds and increasing $E_{H\text{-}bond}$. The resulting lowest-energy structure reveals optimal packing (minimal E_{VDW}) and solvent exposure (minimal E_{solv}); nonpolar side chains are less accessible to polar solvent as compared with the initial α-helical structure. In vacuum, stable conformations are tightly packed and demonstrate low values of E_{VDW}. A significant contribution of $E_{H\text{-}bond}$ is caused by the formation of side chain/side chain and side chain/backbone H-bonds.

Analysis of the distribution of side chain dihedral angles χ^1 leads to the following conclusions. 1) A total number of rotamers for side chains of BRh-A is maximal in vacuum, where several rotameric states were observed for residues Trp-10, 12, Ile-11, Leu-15, 19, 22, 28, Thr-24, Tyr-26, Phe-27, Val-29. 2) In cyclohexane and water all residues (except Leu-22 in cyclohexane) have only one χ^1-rotamer. We should note, however, that analysis of all accepted conformers shows a somewhat larger number of stable

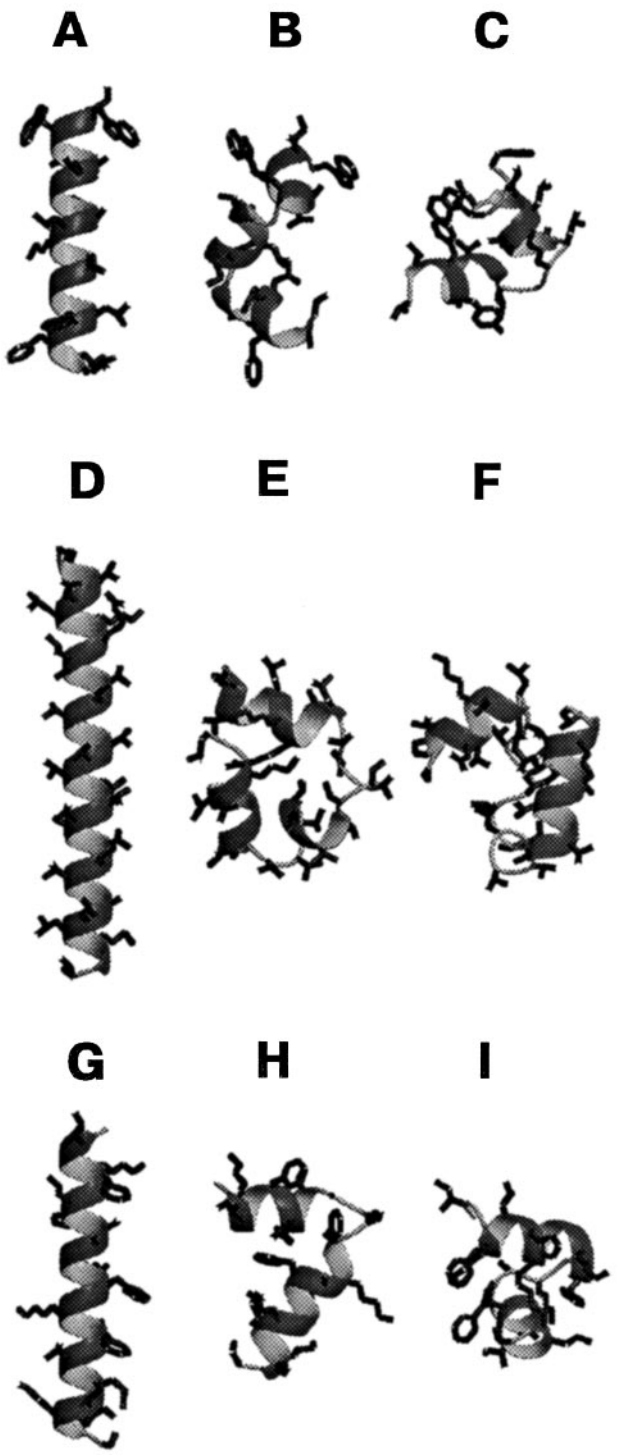

FIGURE 2 Ribbon representation of the lowest-energy conformers of membrane segments BRh-A, SP-C, and magainin2 obtained as the result of variable-temperature Monte Carlo simulations in different environments. (*A–C*) Lowest-energy conformers of BRh-A obtained with the following sets of atomic solvation parameters: ASP_{gc}, ASP_{gw}, and in vacuum, respectively; (*D–F*) The same for SP-C; (*G–I*) The same for magainin2. The molecules are depicted with the N terminus up and C terminus down.

rotamers in cyclohexane than in water (data not shown). 3) Rotameric states for Trp-12 are different in polar and nonpolar solvents. To check how correct the rotameric states extracted from MC simulations in nonpolar environments are, we compared them with the rotamers obtained from NMR data in organic solvent and in micelles (Pervushin and Arseniev, 1992; Table 2). It is seen that the calculated rotamers agree fairly well with those observed by NMR. Both experimental and calculated structures are in α-helical conformation, and this is important for comparison of the rotamers because conformation of the side chain depends on that of the backbone. Interestingly, the starting structure of BRh-A used in the simulations was derived from electron cryomicroscopic data, and the initial side chain conformations deviated greatly from those found by NMR. But in the result, the only difference between the side chain conformations obtained with our solvation model and by NMR is a χ^1-rotamer of Trp-12. The calculated rotamer (g^-) rarely occurs in the middle of α-helices. Most likely, such a disagreement can be explained by proximity of this residue to the N-terminus of BRh-A used in modeling, whereas the experimental data were obtained for fragment 1–36 of BRh, where Trp-12 was displaced far away from the terminus.

To check sensitivity of the results to the number of ASP types (*M*, see details in accompanying paper), we have applied the same protocol to simulate BRh-A with only five instead of eight ASP types derived from gas-cyclohexane energies of transfer. Analysis of the low-energy conformers demonstrated that in general the results resemble those obtained with $M = 8$, although the convergence was slower. Thus, χ^1-rotamers of Leu-22 and Val-29 were found corresponding to the electron microscopic, but not NMR, structure. This confirms our choice of the optimal number of ASP types $M = 8$ in the solvation model.

Transmembrane segment B of bacteriorhodopsin

The backbone and side chain dihedral angles of fragment Lys-40–Gly-65 of BRh obtained in the MC conformational search are presented in Fig. 3. It is seen that, as in the case of BRh-A, the α-helix is well-retained in nonpolar solvent: only Val-49, which is followed by Pro, is not in the helical but in bend ("S") conformation. This causes a kink of the helix with the angle $\sim$32° (27° in the NMR-derived models). Calculated structure in water is quite similar: the helix does not unfold during long-term MC run, except Val-49 (identifier "S" for the secondary structure). At the same time, fluctuations of the backbone dihedrals are somewhat higher in water in comparison with cyclohexane. In both solvents the lowest-energy conformers reveal approximately the same values of ASA: 3036 Å^2 in cyclohexane and 2997 Å^2 in water. On the contrary, the lowest-energy structure in vacuum is different: the helix is broken on residues Ile-45–Val-49 (secondary structure: "TTSS-"), and the two helical fragments Ala-39–Ala-44 and Pro-50–Leu-62 are tightly packed, resulting in compact structure with considerably lower accessible surface (2525 Å^2). The peptide's termini are also partially unfolded. Comparison of χ^1-rotamers of BRh-B in cyclohexane with those observed

TABLE 1 Energetic characteristics of the conformers of transmembrane segment BRh-A obtained in variable-temperature Monte Carlo simulations in different environments

N_α	E_{total}^{min}	E_{total}^{max}	ΔE	$\bar{E}$	σ_E	E_{VDW}	E_{solv}	$E_{H\text{-}bond}$
				Nonpolar solvent: ASP_{gc}*				
18	−196.8	−167.9	28.9	−193.4	5.8	−137.9	−37.4	−35.4
				Water: ASP_{gw}*				
18	−148.8	−143.4	5.4	−147.1	2.2	−127.2	−14.1	−28.8
17	−154.6	−150.6	4.0	−153.2	1.5	−130.2	−20.9	−27.0
16	−168.0	−155.2	12.8	−166.5	2.0	−142.6	−23.5	−24.5
				*Vacuum**				
11	−156.9	−145.7	11.2	−151.2	3.7	−152.3	0.0	−30.6
5	−156.4	−156.4	0.0	−156.4	0	−162.2	0.0	−22.9
10	−156.3	−151.0	5.3	−153.7	1.9	−154.3	0.0	−29.1
12	−155.2	−153.1	2.1	−154.6	0.9	−159.6	0.0	−23.1

Only low-energy conformers are shown. N_α, number of residues in α-helix; E_{total}^{min}, E_{total}^{max}, minimal and maximal energies of total energy of conformers with given secondary structure (given N_α); $\Delta E = E_{total}^{max} - E_{total}^{min}$; $\bar{E}$ and σ_E, mean and standard deviation of the energy distribution; E_{VDW}, E_{solv}, $E_{H\text{-}bond}$, van der Waals, solvation, and H-bonding contributions into the total energy of the lowest-energy conformer with given N_α.
*Data correspond to the minimal-energy conformer with given N_α.

by NMR spectroscopy in micelles (Table 3) demonstrates fairly good agreement between the calculated and experimental structures for most of the residues, except Met-60 and Tyr-64. The main difference is a larger flexibility of the experimental structures, where multiple rotameric states were found for a number of residues.

As in the case of BRh-A, the lowest-energy conformers of BRh-B reveal several side chain/backbone H-bonds that involve H^γ and O^γ atoms of Ser-35, Ser-59, Thr-47, and Thr-55. Interestingly, polar side chains of such residues as Ser and Thr are often found on lipid-exposed faces of TM segments in integral membrane proteins (e.g., Samatey et al., 1995), where they are stabilized by forming H-bonds with the helix backbone. As follows from NMR data (e.g., Pervushin and Arseniev, 1992; Lomize et al., 1992), similar effects are also observed for monomeric peptides in micelles. The results obtained with our solvation model also reproduce this phenomenon: the most energetically favorable conformers of BRh-A and BRh-B in a membrane-like environment demonstrate stabilization of OH groups of Ser and Thr via H-bonding to the backbone.

TABLE 2 χ^1 Side chain rotamers of transmembrane segment BRh-A from variable-temperature MC simulations with atomic solvation parameters for gas-cyclohexane (ASP_{gc}) and NMR data

	χ^1-rotamers*		
	MC#	NMR	
Residue	ASP_{gc}	Chl/Met§	SDS§
Trp-10	t	t	t
Ile-11	g^+	g^+	g^+
Trp-12	g^-	t	t
Leu-13	t	t	t
Leu-15	t	g^+	t, g^+
Thr-17	g^+	g^+	g^+
Leu-19	t	t, g^+	t
Met-20	t	t, g^+	g^+
Leu-22	g^+, t	g^+	t
Thr-24	g^+	g^+	g^+
Leu-25	t	t, g^+	t
Tyr-26	t	t	t
Phe-27	t	t	t
Leu-28	t	t, g^+	t, g^+
Val-29	t	t	t

*The g^+, g^- and t χ^1-rotamers around the C_α–C_β bond correspond to χ^1-torsion angle intervals $-60 \pm 30°$, $60 \pm 30°$ and $180 \pm 30°$, respectively.
#This work. Results of MC simulations with gas-cyclohexane (gc) ASPs. The rotamers were extracted from a set of lowest-energy conformers (50% of all accepted conformers).
§The rotamers in fragments 1–36 of bacteriorhodopsin in chloroform/methanol (Chl/Met) and SDS micelles (SDS) determined from NMR data (Pervushin and Arseniev, 1992).

To summarize, the structures of BRh-A and BRh-B calculated using parameter set ASP_{gc} reveal reasonable overall agreement with the conformations obtained in micelles and $CH_3OH/CHCl_3$ solvent by NMR spectroscopy. The implicit solvation model with membrane-mimetic parameters reproduces a strong helix-promoting effect induced by an apolar environment known from the experiment (e.g., Deber and Goto, 1996). In addition, unlike other simulations of peptides in bilayers (e.g., Milik and Skolnick, 1993; Ducarme et al., 1998), in our model the molecules were not restrained to any predefined conformation (e.g., α-helix), and the effect of solvent on intramolecular energy terms was taken into account. The approach could be efficiently employed for peptides that traverse a bilayer and stay immersed in the nonpolar core of the membrane: their conformational, H-bonding, etc. properties (some deviations from the experiment might occur on the termini exposed to polar environment) are well described by the ASP_{gc} set.

Surfactant lipoprotein

SP-C is a 35-residue polypeptide essential for the function of surfactants used for therapy of infant respiratory distress (Johansson et al., 1995). It has been shown that SP-C could insert into lipid bilayers in TM orientation (Johansson et al., 1994, 1995 and references therein). As follows from NMR

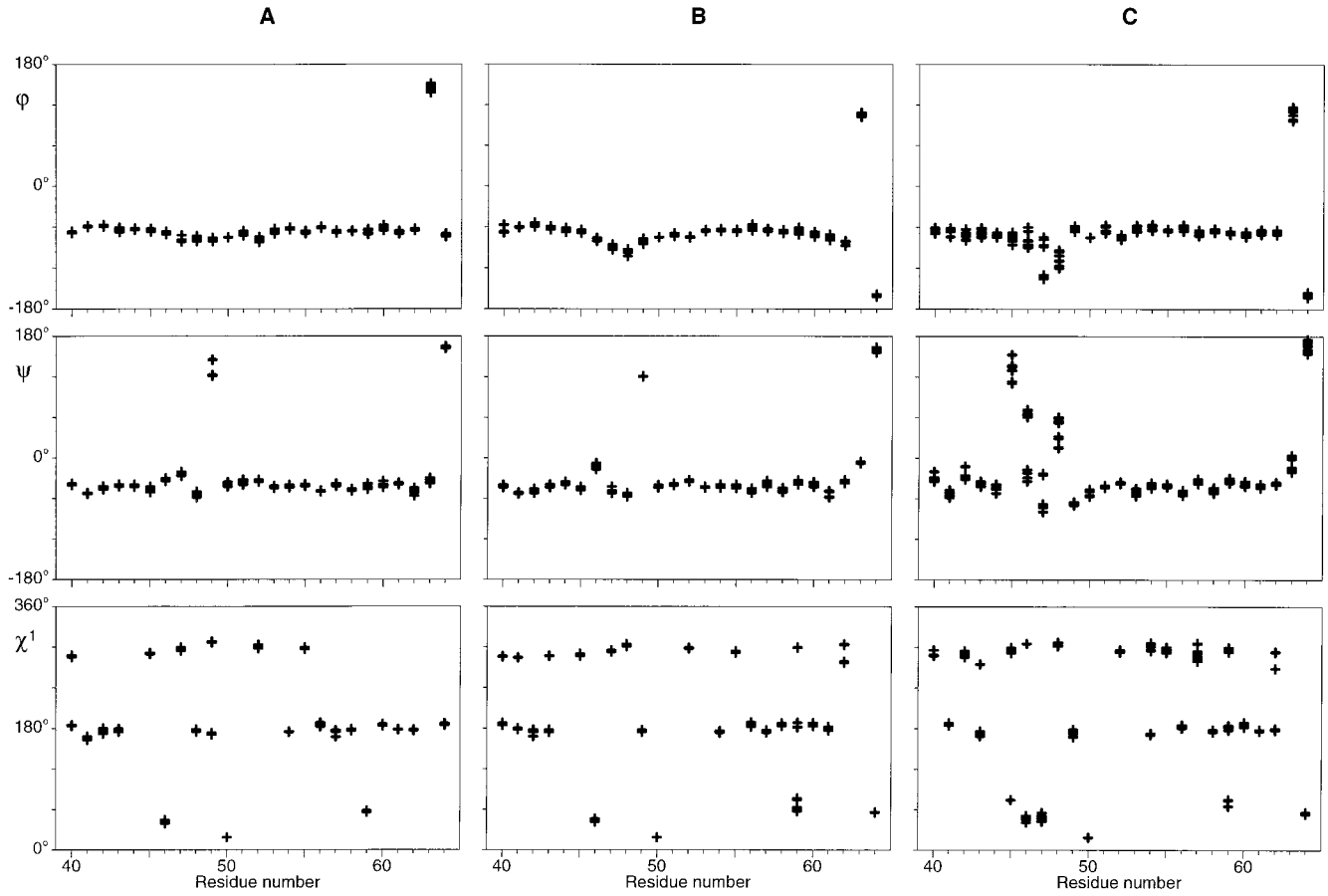

FIGURE 3 Dihedral angle distribution during Monte Carlo simulations of transmembrane segment BRh-B at variable temperature with the following sets of atomic solvation parameters (ASP): (*A*) ASP_{gc}; (*B*) ASP_{gw}; (*C*) in vacuum. φ, ψ, and χ^1 angles in 50% of low-energy accepted conformations are plotted versus the residue number.

data (Johansson et al., 1995), in micelles or mixed organic solvent it forms a highly regular α-helix. Because SP-C contains stretches of seven and four consecutive valine residues that have prominent β-sheet forming propensities in water, it is interesting to assess its conformation in environments of different polarity. These questions were recently addressed in the work of Kovacs et al. (1995) who explored behavior of the α-helical SP-C via MD simulations in explicit solvent environments of chloroform, methanol, and water. It was found that the α-helix is stable in water and methanol, while some destabilization appears in chloroform. Also, the polyvalyl part (residues 15–21) remains intact even at elevated temperature, and the valines do not disrupt the α-helical conformation. Therefore, availability of experimental indications on the secondary structure in micelles and results of explicit solvent simulations make SP-C a convenient model to check our method and investigate helix-forming propensities in different environments in detail. To accomplish this, two series of simulations were performed. We explored the peptide's conformational space in the vicinity of the α-helical conformation using either variable-temperature MC search with minimization of the conformers or constant-temperature MC runs without minimization.

Variable-temperature MC conformational search

The results of the variable-temperature MC run with ASPs for nonpolar and polar solvents, as well as in vacuo, are shown in Figs. 2, *D–F*, 4, and 5. As seen in Fig. 2, the lowest-energy conformers found with ASP_{gc} and ASP_{gw} sets and in vacuo are remarkably different. The initial α-helical conformation is stable in hydrophobic media (Figs. 2 *D*, 4 *A*), while in water and vacuum it is distorted in the central part. In the lowest-energy structures found in water, nonhelical conformation was attributed to residues Leu-14–Val-24. Interestingly, unfolding of initial all-helical structure in water occurs already at the stage of minimization before executing MC protocol and then it extends, leading to large conformational changes of SP-C. As was reasonable to expect based on low helix-forming propensities of valine in aqueous solution, in water the helix is broken in the region containing seven consecutive valine residues, and the remaining two α-helical segments are packed together, leading to significantly decreasing total ASA. In the lowest-energy conformers the values of total ASA in cyclohexane, water, and vacuum are 3086, 2645, and 2338 $Å^2$, respectively. In vacuum the helix is not completely destroyed

TABLE 3 χ^1 Side chain rotamers of transmembrane segment BRh-B from variable-temperature MC simulations with atomic solvation parameters for gas-cyclohexane (ASP_{gc}) and NMR data

	χ^1-Rotamers*	
Residue	MC[#]	NMR[§]
Lys-40	g^+	g^+, t
Lys-41	t	g^+, t
Phe-42	t	t
Tyr-43	t	t
Ile-45	g^+	g^+
Thr-46	g^-	g^-
Thr-47	g^+	g^+, g^-
Leu-48	t	g^+, t
Val-49	t, g^+	t
Ile-52	g^+	g^+
Phe-54	t	t
Thr-55	g^+	g^+
Met-56	t	g^+, t
Tyr-57	t	t
Leu-58	t	g^+, t
Ser-59	g^-	g^+, g^-, t
Met-60	t	g^+, t
Leu-61	t	t
Leu-62	t	g^+, t
Tyr-64	t	g^+

*The g^+, g^-, and t χ^1-rotamers around the C_α–C_β bond correspond to χ^1-torsion angle intervals $-60 \pm 30°$, $60 \pm 30°$, and $180 \pm 30°$, respectively.

[#]This work. Results of MC simulations with gas-cyclohexane (gc) ASPs. The rotamers were extracted from a set of lowest-energy conformers (50% of all accepted conformers).

[§]The rotamers in fragments 40–64 of bacteriorhodopsin in SDS micelles (SDS) determined from NMR data (Lomize et al., 1992).

between valines 15–21: it is partially transformed into 3_{10}-helix and reveal a break on residues Val-15 and Val-16. In this case, loss of the helical structure is also observed for 4-residue polyvalyl segment 24–26. These results are different from MD simulations in explicit solvents (Kovacs et al., 1995), where initial α-helical conformation was found to be well-retained in water and, to a lesser degree, in chloroform. Unfortunately, no experimental structural data on SP-C in water are available, and thus it is difficult to judge which model better reproduces the peptide's behavior in aqueous solution. However, numerous experimental (e.g., Lyu et al., 1991) and computational (e.g., Tobias and Brooks, 1991) data (including those obtained with explicit and implicit solvents) point to destabilization of the α-helix by valine residues, and therefore agree with our results.

Fig. 4 displays dihedral angles φ, ψ, and χ^1 of the conformers accumulated during the simulation. As seen from the analysis of φ and ψ angles, their deviations from standard values for α-helix increase in the order cyclohexane $\rightarrow$ water $\rightarrow$ vacuum. In the nonpolar environment the deviations of angle ψ were observed only for Leu-14 (N-terminal Cys-6 was not counted) although corresponding conformers were not the most energetically favorable. In water, large deviations of φ and ψ were obtained for residues 14–24, whereas α-helical conformation was retained for residues 7–15 and 25–34. In vacuum, stable α-helix was detected for residues 7–14, 20–21, and 27–33, and helix 3_{10} was found for residues 17–19. Interestingly, helix distortion in water and vacuum was observed for stretches of valines in the central part of SP-C, although in nonpolar media this part was observed only in α-helical conformation.

Additional insight into structural properties of SP-C in membrane-mimetic media is provided by analysis of distributions of resulting conformers over their energies (E). Fig. 5 shows total energies of conformers combined into several groups according to their helicity (N_α) and structural similarity (in terms of root-mean-square deviation (r.m.s.d.) values). It is seen that the rod-like helical structures able to traverse the bilayer and containing either 28 (groups 1 and 2) or 27 (group 3) residues in the α-helix demonstrate the lowest energies on a wide interval ($\sim$12 kcal/mol). The neighboring group of conformers (group 4, $N_\alpha = 27$) is separated by the energy gap of $\sim$3.5 kcal/mol and reveal a pronounced kink on residue Leu-9. This structure is realized only within small intervals of E ($\Delta E \approx 1.7$ kcal/mol). Other structures with $N_\alpha = 26$, 25, 24, etc. have energies $\sim$5 kcal/mol higher than the maximal-energy conformers from groups 1–3.

In general, the numbers of χ^1-rotamers of SP-C in nonpolar and polar media are close to each other, and in vacuum it is higher (Fig. 4). At the same time, the rotameric states of valine residues are different in cyclohexane and water. Thus, in cyclohexane the 180° rotamer is noticeably populated (except Val-17). This agrees well with the experimental data on SP-C in apolar organic solvent (Johansson et al., 1994). Small fractions of $+70°$ and $-60°$ rotamers were also found for Val-15, Val-20, and Val-23. In water, χ^1 angles of valines 15–21 fall close to $-60°$, although Val-15 and -19 also have $+60°$ rotamers. On the contrary, the second stretch of valines (23–26), Val-8, and Val-28 have dominant rotamers with χ^1 angles near 180°. In vacuum, most valines demonstrate two rotamers. Some differences in rotameric states in nonpolar and polar solvents were observed for leucines 14, 22, and 32: in cyclohexane, χ^1 angles for all of them are 180°, while in water they are $-60°$, $-60°$, and $180°/-60°$. Interestingly, rotamer distributions on the N-terminus (residues 6–13) are almost similar in both environments.

The side chain rotameric states of SP-C reported above are different from those obtained in the result of MD simulations with explicit solvents (Kovacs et al., 1995). Thus, for the 1-ns simulations, χ^1 angles for valines 15–21 were similar in nonpolar (chloroform) and polar (methanol) solvents, revealing only slight dominance of the $-65 \pm 5°$ rotamer, and the 180° rotamer was populated only for Val-8 and Val-15 in the chloroform simulation. For these two valines we found the same rotamers in cyclohexane, although the MD data for other valines agree with our results obtained in water, but not in cyclohexane. In general, the results of MD simulations with explicit solvent and MC conformational search with ASPs are close to each other, except the valines. Thus, in the case of MD, conformational

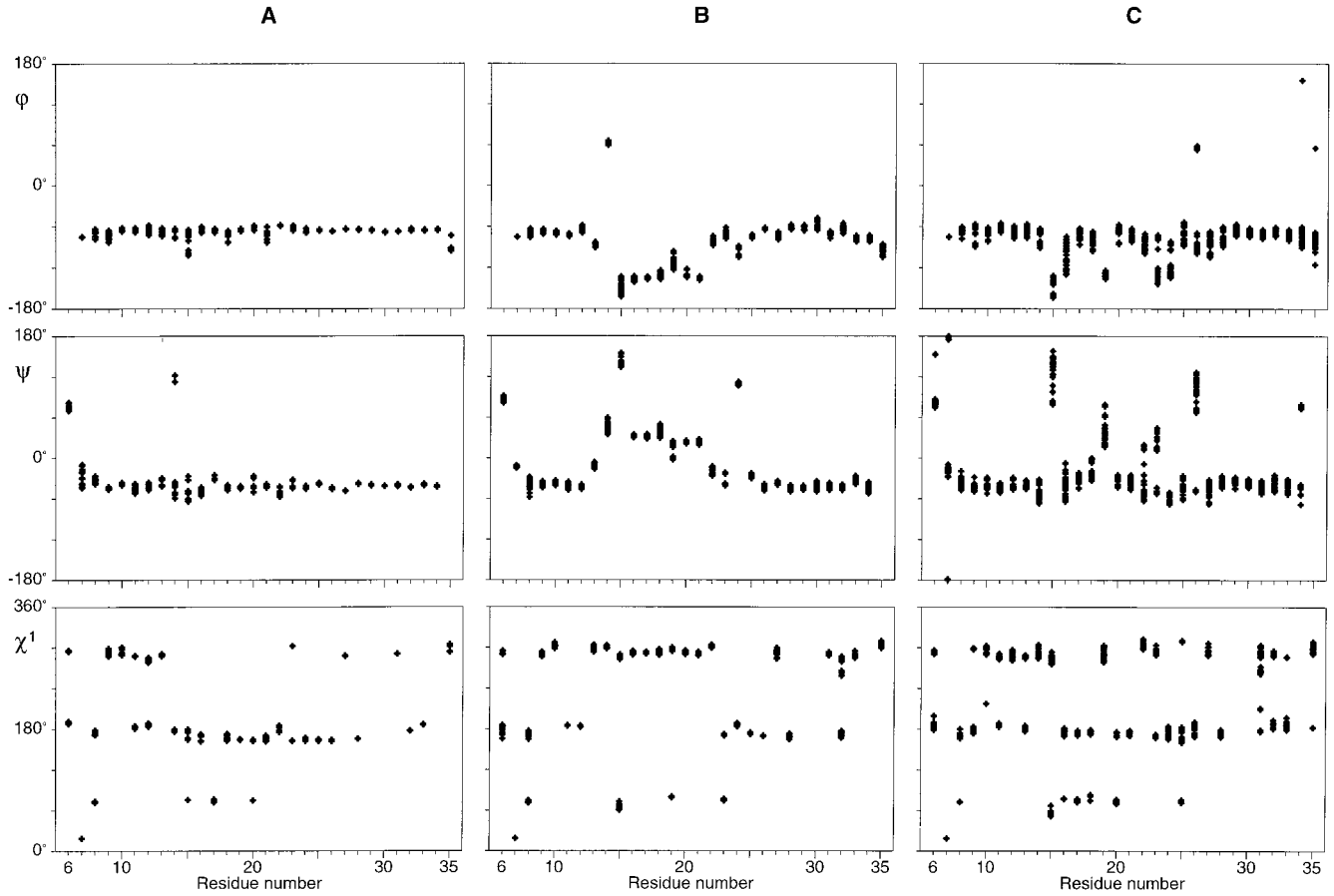

FIGURE 4 Dihedral angle distribution during Monte Carlo simulations of SP-C at variable temperature with the following sets of atomic solvation parameters (ASP): (*A*) ASP_{gc}; (*B*) ASP_{gw}; (*C*) in vacuum. φ, ψ, and χ^1 angles in 50% of low-energy accepted conformations are plotted versus the residue number.

freedom of the valine side chains is larger in nonpolar than in polar solvent, whereas our MC results show that the corresponding numbers of rotamers are quite similar. It should be noted, however, that the rotamers found in this work were subjected to energy minimization, while those reported in the MD study were not.

Constant-temperature MC simulations

Exhaustive conformational search in water shows that the initial α-helical structure does not represent the deepest minimum on the potential energy hyper-surface. Raising temperature combined with energy minimization permits the system to migrate into the lower energy subspace, which does not correspond to all-helical structure. It is interesting to note that these regions of conformational space were not explored in MD simulations with explicit solvent where the initial α-helical structure was found to be stable. We suppose this is due to limited length of MD trajectory (1 ns) used by Kovacs et al. (1995), which did not allow the system to pass the energy barriers separating all-helical states from the others. To check behavior of the α-helix of SP-C in the vicinity of all-helical conformation and to compare these data with those obtained using explicit solvent models in more detail, we performed MC simulation in cyclohexane and water under "mild" conditions (at constant temperature and without energy minimization) resembling those used in explicit solvent MD.

Fig. 6 shows distribution of backbone and side chain dihedral angles of the conformers obtained in MC simulations with ASPs imitating cyclohexane (*A*) and water (*B*). In both solvents the initial α-helix was stable, but fluctuations of φ and ψ angles are somewhat larger in water than in cyclohexane. Conformational mobility of side chains is quite similar in both cases, although the total number of different rotameric states is a bit higher in water (Fig. 7). Thus, maximal r.m.s.d. values between coordinates of backbone atoms of the starting and successive conformers obtained in the MC runs after 100 steps were 0.65 and 0.99 in cyclohexane and water, respectively. Although the helix was also stable in 1 ns MD, somewhat larger r.m.s.d. values were obtained in chloroform than in water. The largest fluctuations of the backbone and side chain dihedrals were always observed in the C-terminal part. In general, results of MC simulation of SP-C agree better with MD data than the results of the more exhaustive variable-temperature MC

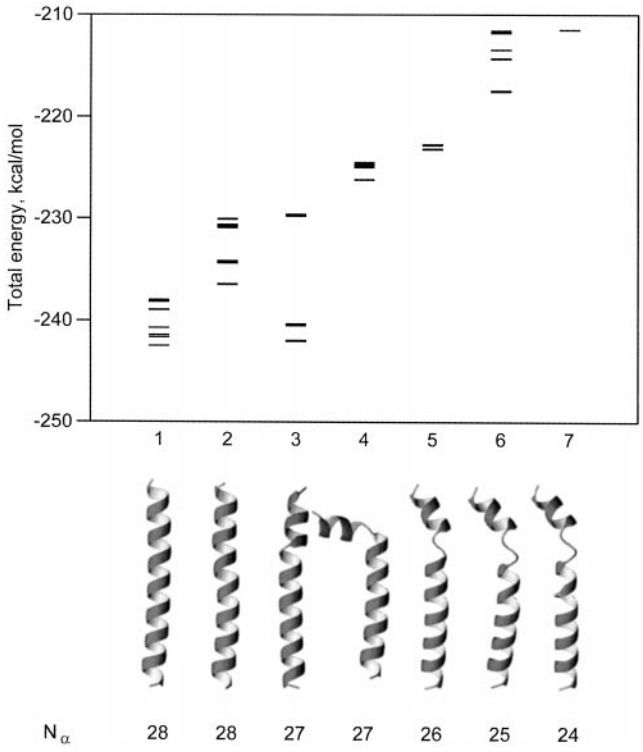

FIGURE 5 Low-energy conformers of SP-C obtained in the result of variable-temperature Monte Carlo conformational search in cyclohexane. *Top:* total energy versus α-helical content (N_α). The conformers are grouped according to r.m.s.d. values (taken over backbone atoms) with the lowest-energy structure. *Bottom:* ribbon diagrams of the conformers (calculated for the minimal-energy structure from the corresponding group). Numbers 1–7 along the *x*-axis indicate the groups of conformers. The molecules are depicted with the N terminus up and C terminus down.

conformational search presented in the previous section. It seems that neither 14,000 MC steps nor 1 ns MD trajectories were unable to trap the low-energy regions of the conformational space that are accessible to SP-C in water and which correspond to distorted α-helical structure.

Thus, ASPs developed in this study better retain α-helical conformation of valine-containing peptides in nonpolar media as compared to simulations with explicit solvent. (We should remind that MD data were collected in chloroform, while in our model we employed ASPs for cyclohexane which is more hydrophobic.) However, in water our model demonstrates considerably lower helix-forming propensities for Val residues than those obtained with explicit solvent. These inferences are in good accord with the experimental data on membrane-promoting α-helix stability (Deber and Goto, 1996) and helix-distortion in aqueous solutions (e.g., Lyu et al., 1991; Tobias and Brooks, 1991).

Magainin2

Magainin2 is a 23-residue antimicrobial peptide from *Xenopus* skin that binds to the cell membrane (Zasloff, 1987). The peptide contains many charged and polar residues that are rarely found in hydrophobic membrane environment. NMR studies have shown that magainins reveal high helical content in detergent micelles and are unfolded in aqueous solution (Bechinger et al., 1991; Bechinger, 1997). Recent experimental studies (see Shai, 1995 for a review) suggest that peptides of this class bind parallel to the membrane surface and do not penetrate deeply into the hydrophobic core ("carpet-like" model). The limited degree of self-association might also occur, leading to formation of transbilayer pores ("barrel-stave" model). Although the mode of action of magainins is not yet well understood, it strongly depends on the peptide-membrane interactions (Shai, 1995), and therefore studies of solvent effects on structure and behavior of magainin2 are of particular importance.

MC simulations of magainin2 in different solvents and in vacuo show similar tendencies to the peptides considered above. Thus, the lowest-energy conformers in nonpolar media are totally α-helical (Fig. 2 *G*), while those in water ($N_\alpha = 17$) and in vacuum ($N_\alpha = 16$) are unordered in the middle part and near the termini (Fig. 2, *H* and *I*). α-Helix in cyclohexane is retained within a wide interval of energies ($\Delta E \approx 22$ kcal/mol). In water, conformers with $N_\alpha = 21$, 20, and 17 were observed. Corresponding values of ΔE are 7.1, 8.5, and 10.4 kcal/mol, respectively. As for other peptides described above, multiple conformational states with close energies but different secondary structure ($16 \leq N_\alpha \leq 21$) were obtained in vacuum. Finally, very similar tendencies in behavior of the backbone and side chain dihedrals were observed for magainin2 (data not shown) as compared with BRh-A, BRh-B, and SP-C. The principal result for magainin2 is in qualitative agreement with the experimental data, i.e., in the nonpolar environment the amphiphilic peptide containing many charged groups retains α-helicity and does not fold into compact structure.

CONCLUSIONS

MC simulations were employed to explore conformational space of several membrane-binding peptides in environments of different polarity and in vacuum. The solvent effects were treated using an ASP-based implicit solvation model. Nonpolar solvent imitating membrane environment and aqueous solution were represented by ASPs for gas-cyclohexane and gas-water transfer, respectively. It is important that unlike many previous studies, the simulations were done for all-atom models of peptides that were not restrained to any predetermined conformation. The results emphasize that the α-helical conformation is promoted by nonpolar solvent and exists in a wide energy range. The lowest-energy structures found for BRh-A and BRh-B agree fairly well with those derived in NMR experiments. Conformational properties of SP-C and magainin2 in the membrane-like environment were also found to be in accord with available experimental data.

On the contrary, simulations in water reveal helix distortion for the peptides under study. Our results for SP-C do not confirm preference of all-helical structure for SP-C in

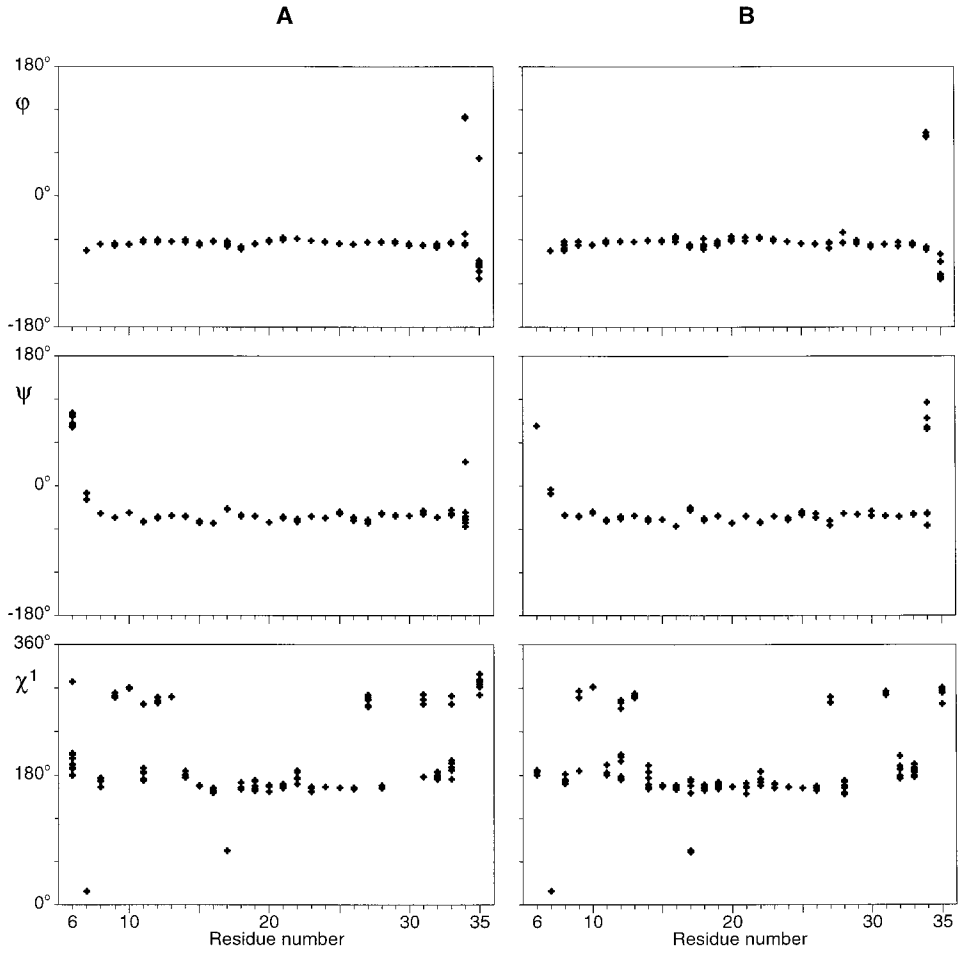

FIGURE 6 Dihedral angle distribution during Monte Carlo simulations of SP-C at 300 K with the following sets of atomic solvation parameters (ASP): (*A*) ASP_{gc}; (*B*) ASP_{gw}; (*C*) in vacuum. φ, ψ, and χ^1 angles in 50% of low-energy accepted conformations are plotted versus the residue number.

water, which was reported in an MD study with explicit solvent (Kovacs et al., 1995); although MC simulation at constant temperature shows retention of the helical conformation, exhaustive MC search with variable temperature reveals that the helix might be unfolded in the middle part (corresponding to polyvalyl segment Val-15–Val-21), and the structure is tightly packed, thus reducing its exposure to polar solvent. This observation is consistent with known helix-destabilizing properties of valines in aqueous solution. Unlike explicit solvent calculations, no significant restriction of χ^1-rotamer sampling was obtained in a nonpolar solvent compared to a polar one. The solvation model proposed here gives more realistic results than simulations in vacuum, where the helical structure undergoes rapid distortion and demonstrates large conformational freedom for the side chains. In addition, multiple conformational states (local minima) separated by low-energy barriers and having different secondary structure and packing were found in vacuum. A conclusion was made that simulations in vacuo could not be used to model membrane-bound peptides.

We should outline that the restricted MC search presented here permits exploring only the potential energy hypersurface in the vicinity of starting experimentally observed structure. Recently, we have performed detailed exploration of the conformational space of BRh-A in nonpolar solvent, starting from random coil conformation (Efremov et al., in preparation). During the simulation, numerous local minima corresponding to unordered structures and conformations containing helical segments of different length were trapped. In the result, the lowest-energy conformers were found to be all-helical and correspond well to the structure in micelles obtained by NMR. This implies that the ASP approach provides a realistic description of environmental effects induced by a membrane and permits selection of the native state, among many others.

Our current work is pursued to study helix-forming properties of other peptides in membrane-like surroundings through detailed exploration of their conformational space in MC simulations. Hopefully, this will provide important insight into the structural behavior of peptides in lipid bilayer and will permit development of algorithms designed to structure prediction of membrane proteins based on their sequences. Simulations in bulk solvent do not address questions concerning adsorption and/or insertion of peptides, water/bilayer partitioning, and peptides' orientation with respect to the bilayer. For this to be done the heterogeneous nature of membranes, namely polar layers separated by a hydrophobic core, should be taken into account. The work combining ASPs for cyclohexane and water in the frame-

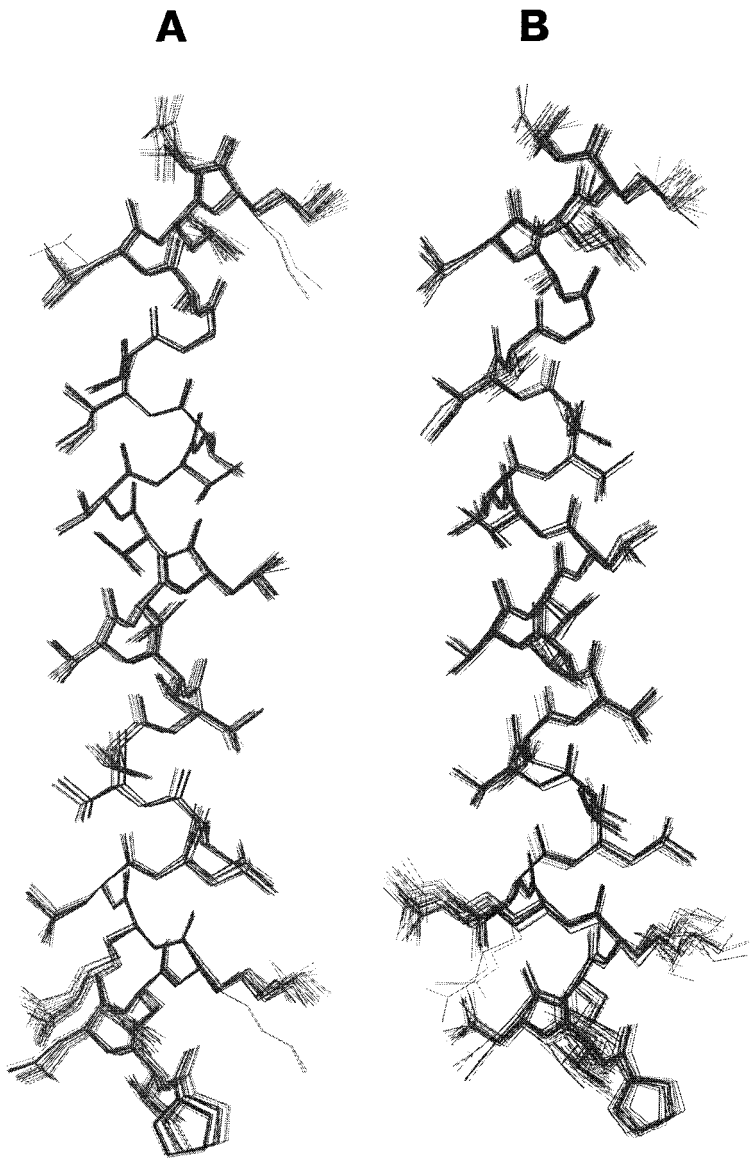

FIGURE 7 Superposition of coordinates of SP-C obtained in the results of Monte Carlo simulations at 300 K starting from the same initial structure with the atomic solvation parameters (ASP): (*A*) ASP_{gc}; (*B*) ASP_{gw}. Superposition was done over backbone atoms. The conformations were extracted after 100 consecutive Monte Carlo steps. The molecules are depicted with the N terminus up and C terminus down.

work of such a three-phase solvation model is in progress now.

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