A Solvent Model for Simulations of Peptides in Bilayers. I. Membrane-Promoting α-Helix Formation

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ABSTRACT We describe an efficient solvation model for proteins. In this model atomic solvation parameters imitating the hydrocarbon core of a membrane, water, and weak polar solvent (octanol) were developed. An optimal number of solvation parameters was chosen based on analysis of atomic hydrophobicities and fitting experimental free energies of gas-cyclohexane, gas-water, and octanol-water transfer for amino acids. The solvation energy term incorporated into the ECEPP/2 potential energy function was tested in Monte Carlo simulations of a number of small peptides with known energies of bilayer-water and octanol-water transfer. The calculated properties were shown to agree reasonably well with the experimental data. Furthermore, the solvation model was used to assess membrane-promoting α-helix formation. To accomplish this, all-atom models of 20-residue homopolypeptides—poly-Leu, poly-Val, poly-Ile, and poly-Gly in initial random coil conformation—were subjected to nonrestrained Monte Carlo conformational search in vacuo and with the solvation terms mimicking the water and hydrophobic parts of the bilayer. All the peptides demonstrated their largest helix-forming tendencies in a nonpolar environment, where the lowest-energy conformers of poly-Leu, Val, Ile revealed 100, 95, and 80% of α-helical content, respectively. Energetic and conformational properties of Gly in all environments were shown to be different from those observed for residues with hydrophobic side chains. Applications of the solvation model to simulations of peptides and proteins in the presence of membrane, along with limitations of the approach, are discussed.

INTRODUCTION

Membrane domains in proteins are of prime importance for many cell processes. Often, they are organized as assemblies of polypeptide segments interacting with the lipid bilayer and constituting a functionally active and finely regulated biological machine involved in ion and molecular transport across the membrane, cell communication, signaling, etc. Studies of membrane-bound segments are thus essential for understanding structure-function relationships of membrane proteins. At the same time, high-resolution structural information about them is scarce because of limitations of modern experimental techniques (see Walker and Saraste, 1996, for a review). Given these difficulties, the development of theoretical models for membrane proteins seems to be indispensable.

A number of simulations of membrane proteins and peptides have been performed using the force field methods. Thus, molecular dynamics (MD) and Monte Carlo (MC) protocols (sometimes combined with simulated annealing) were employed to refine (Parker et al., 1992) and predict (Jähnig and Edholm, 1992; Chou et al., 1992; Adams et al., 1996) the structure of intramembrane domains. The main difficulty of these calculations is a necessity to take into account solvent effects. Depending on the way of treatment of such effects, computational studies of peptides directed to assessment of their conformations and energetics in membranes can be subdivided into the following groups: i, studies in vacuo or with a uniform dielectric model of solvent; ii, explicit solvent simulations; and iii, calculations with simplified potentials imitating a membrane.

In studies of group i, properties of residues near the protein surface are not well-described, thus leading to missing important details in the representation of interactions between the protein and its environment. In addition, such simulations neglect the hydrophobic effect playing a dominant role in the determination of conformation and stability of membrane proteins (Wang and Pullman, 1991; Jacobs and White, 1989). Group ii includes calculations in bulk nonpolar solvents (e.g., De Loof et al., 1992; van Buuren and Berendsen, 1993; Gerstein and Lynden-Bell, 1993; Kovacs et al., 1995; Efremov and Vergoten, 1995; Peters et al., 1996). A number of protein simulations in explicit membranes were also reported (e.g., Xing and Scott, 1989; Wang and Pullman, 1991). Although using an explicit solvent provides a solution to the problem, such calculations require very large amounts of computer time and, therefore, still cannot be applied efficiently even to medium-size membrane moieties (e.g., assemblies of four to five transmembrane (TM) α-helices). In addition, such methods involve convergence problems and often lack precision in describing hydrophobic interactions between a protein and its surroundings. As pointed out by Edholm and Jähnig (1988), the environmental effects result from small differences between strong interactions of protein with water and lipid molecules. Correct description of such interactions

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demands precise determination of parameters in van der Waals and electrostatic energy terms. Finally, while numerous simulations of hydrated lipid bilayers have been reported (see Pastor, 1994 for a review), an adequate choice of the all-atom membrane model still is not straightforward.

A promising alternative (iii) lies in employment of either simplified models of a bilayer or membrane-mimicking potential added to the energy of the system. Such methods are sufficiently less computationally expensive than explicit solvent calculations and, therefore, are able to address questions about structure and function of membrane proteins on rather larger time scales. Studies of proteins in simplified membrane models were described by Roux and Karplus (1994), Baumgärtner (1996), and others. Often, the hydrophobic core of a membrane is modeled by Lennard-Jones hydrocarbon-like particles, a polarizable cubic lattice with low dielectric permeability (reviewed in Roux and Karplus, 1994), or by a monolayer of hard parallel cylinders representing the lipid chains (Baumgärtner, 1996). Among other properties, these models permit investigation of orientational order and lateral density fluctuation of the lipid matrix, which are important for partitioning and α-helix formation of TM peptides.

In a number of studies the membrane was approximated by introducing an additional solvation term into the potential energy function to represent interaction of a protein with its environment. Usually (Edholm and Jähnig, 1988; Gersappe et al., 1993; Milik and Skolnick, 1993, 1995; Seagraves and Reinhardt, 1995), such potentials are taken dependent on hydrophobic properties of residues and their positions relative to the bilayer. The results obtained provide interesting insights into peptides' behavior in the membrane environment. However, such methodology seems to be somewhat oversimplified because amino acid residues are treated as point "hydrophobic sites" without taking into account the conformation and hydrophobic nature of atoms and/or atomic groups.

A reliable compromise between preserving atomic details, correct assessment of the hydrophobic interactions, and computational cost of simulations appears to be in employment of atomic solvation parameters (ASP). In this type of implicit solvation model the solvent contribution to the potential energy for solute atoms is taken proportional to their solvent-accessible surface area (ASA). The solvation term is as follows (Eisenberg and McLachlan, 1986):

$$E_{\text{solv}} = \sum_{\text{i}=1}^{\text{N}} \Delta\sigma_{\text{i}} ASA_{\text{i}} \qquad (1)$$

where ASA_{i} is the ASA of atom i, $\Delta\sigma_{\text{i}}$ is its atomic solvation parameter, and N is the number of atoms contributing to the solvation energy. Such a technique with ASPs derived either from the experimental octanol-water free energies of transfer for amino acids or from statistical analysis of known protein structures has been applied to globular proteins (Wesson and Eisenberg, 1992; Schiffer et al., 1993; Stouten et al., 1993; von Freyberg et al., 1993; Cummings et al., 1995; Juffer et al., 1995). In these studies the solvation model imitated residue exposure to water and to weakly polar protein interior (approximated by octanol). Some of the results obtained in these applications agree fairly well with the experimental data, and hence provoke a strong interest in employing ASP-based solvation models in simulations of membrane-bound peptides and proteins.

The intention of the present study is to develop ASPs for nonrestrained simulations of full-atom models of peptides and proteins in a membrane-like environment. The work consists of two parts. In the first one, we design the parameters mimicking the nonpolar hydrocarbon core of a membrane and test them, along with the parameters imitating polar solvents, in MC calculations of small peptides with known energies of bilayer-water and octanol-water transfer. Because now there are only a few applications of the membrane solvation models to simulations of proteins in full-atom representation, special attention was paid to the development and critical assessment of ASPs. In addition, questions concerning the influence of solvent polarity on energetic and conformational properties of Leu, Val, and Ile, which are often found in α-helical conformation in the bilayer, are addressed in simulations of 20-residue homopolypeptides poly-Leu, poly-Val, and poly-Ile. In the accompanying paper we report the results of MC simulations in membrane-mimetic environments for several biologically important TM peptides, revealing a wealth of experimental structural information.

METHOD OF CALCULATION

Atomic solvation parameters

The ASPs were obtained for the following systems: gas/water (gw), octanol/water (ow), gas/cyclohexane (gc), octanol/cyclohexane (oc), and gas/octanol (go). The parameters for each of them (ASP of class k, where $k \equiv$ gw; ow, etc.) were derived by solving an overdetermined system of linear equations of the form

$$\Delta G_{\text{j}}^{\text{k}} = \sum_{\text{i}=1}^{\text{N}_{\text{j}}} \Delta\sigma_{\text{i}}^{\text{k}} ASA_{\text{i}}^{\text{k}}, \qquad (2)$$

using an SVD algorithm that is known to provide the most stable solutions (Lawson and Hanson, 1974). Here $\Delta G_{\text{j}}^{\text{k}}$ is the experimental free energy of transfer of amino acid residue (or its side-chain analog) of type j in a system k (taken from Sharp et al., 1991), $\Delta\sigma_{\text{i}}^{\text{k}}$ and $ASA_{\text{i}}^{\text{k}}$ are ASP and solvent-accessible surface areas for atoms of type i in system k, respectively, and N_{j} is a number of atoms in residue j contributing to $\Delta G_{\text{j}}^{\text{k}}$. The linear system (Eq. 2) was solved for various numbers (M) of the ASP types defined as follows: ($M = 4$) C, N/O, S, N^+/O^-; ($M = 5$) C, N/O, S, N^+, O^-; ($M = 6$) C, N, O, S, N^+, O^-; ($M = 7$, a) $C_{\text{aliph.}}$, $C_{\text{ar./x}}$, N, O, S, N^+, O^-; ($M = 7$, b) $C_{\text{aliph./ar.}}$, C_{x}, N, O, S, N^+, O^-; ($M = 8$) $C_{\text{aliph.}}$, $C_{\text{ar.}}$, C_{x}, N, O, S, N^+, O^-; ($M = 9$) $C_{\text{aliph.}}$, $C_{\text{ar.}}$, C_{x}, C_{xx}, N, O, S, N^+, O^-. Here C is all types of carbon, $C_{\text{aliph.}}$ is aliphatic carbon, $C_{\text{ar.}}$ is aromatic carbon, $C_{\text{aliph./ar.}}$ is either aliphatic or aromatic carbon, $C_{\text{ar./x}}$ is either aromatic carbon or carbon attached to a heteroatom, C_{x} is carbon attached to any number ($M = 4 \div 8$) or one ($M = 9$) heteroatom (O, N, S, including those in aromatic rings), C_{xx} is carbon attached to two heteroatoms ($M = 9$), N is uncharged nitrogens, O is uncharged oxygens, N^+ is charged nitrogens, O^- is charged oxygens, and S is sulfur atoms. ASA values (and, therefore,

$E_{solv.}$) for these atoms were estimated by considering them in united-atom presentation, although the other energy terms (see below) were calculated in all-atom presentation. The probe radii for water and octanol were taken to be 1.4 Å. For cyclohexane two probe radii were tested: 1.4 and 3.3 Å. The united-atom ASAs were calculated for extended conformations of corresponding N-acetyl amino acid amides using the FANTOM program (von Freyberg and Braun, 1991), whereas the backbone atoms of amino acid side chains were treated as pseudoatoms. The ASA values are available from the authors (ERG, efremov@nmr.ru) upon request.

MC simulations

Starting all-atom models of Ac-Trp-Leu_m (COO^-) (m = 1, 2, 3, 4), Ac-Gln-X-Ile-NMe (Ac = N-acetyl, NMe = methyl amide, X = Gly, Trp, Ala) peptides as well as 20-residue poly-L-Leu, poly-L-Val, poly-L-Ile, and poly-L-Gly were taken in random conformations built by the FANTOM program. Identical starting conformations were used in simulations of the same peptide with different sets of ASPs. The homopolypeptides were taken with neutral N- and C-termini.

The peptides were subjected to MC simulations in torsion angle space using the FANTOM program. The Metropolis criterion (Metropolis et al., 1953) was used to select conformations during the sampling. The potential energy function was taken in the form

$$E_{total} = E_{ECEPP/2} + E_{solv.} \quad (3)$$

Here the term $E_{ECEPP/2}$ includes van der Waals, torsion, electrostatic, and H-bonding contributions to the potential energy (Némethy et al., 1983). $E_{solv.}$ is a solvation energy (Eq. 1).

For homopolypeptides the following simulation 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, 10 randomly selected dihedral angles were sampled, the step of variation of each dihedral was chosen randomly on the range $-180° \div 180°$, and the current structure was minimized via 100 conjugate gradient iterations. 2) The adaptive-temperature schedule protocol (von Freyberg and Braun, 1991) was employed during 2000 iterations by sampling of five randomly selected dihedrals followed by 150 minimization steps. The initial conformation was the lowest-energy structure found at stage (1). 3) Finally, the protocol similar to (2) but with one dihedral sampled was applied during 1000 MC steps. To assess the influence of a number of model parameters (M) on the results, we have performed MC simulations of 10-residue poly-Leu with ASP_{gc} calculated for M = 5 and M = 8. The peptide was taken in initial random conformation, and the simulation scheme (1) with 1500 MC steps was employed.

In all the calculations distance-dependent dielectric permeability $\varepsilon = 4 \times r$ and spherical cutoff for nonbond interactions (30 Å) were used. The ω angles of peptides were kept fixed in the MC runs. No distance or torsion restraints were employed. Two conformers were considered as identical if, apart from symmetry operation, all torsion angles of both conformers agree to within 1°. Other details of MC protocol can be found in von Freyberg and Braun (1991). Secondary structure, ASAs, and H-bonding patterns were analyzed using the DSSP program (Kabsch and Sander, 1983). Ribbon diagrams of the molecules were produced with the MOLMOL program (Koradi et al., 1996).

Free energies of octanol-water (ΔG_{ow}) or cyclohexane-water (ΔG_{cw}) transfer were estimated for series of Ac-Trp-Leu_m (COO^-) (m = 1, 2, 3, 4) and Ac-Gln-X-Ile-NMe (where X = Gly, Trp, Ala) peptides, respectively. (We should outline that the procedure for calculation of ΔG described below is valid only for small peptides, where the main contribution to ΔG is determined by the solvation term.) For each peptide, two MC simulations with the ASP sets ASP_{gw}, ASP_{gc} (or ASP_{gw}, ASP_{go}) were performed as follows: initial random structures were subjected to energy minimization followed by 1500 MC steps at T = 300 K. At each iteration, one randomly selected dihedral angle was sampled. Other details were the same as described before. Then, the values of ΔG_{ow} and ΔG_{cw} were calculated according to the formulas: $\Delta G_{ow} = \langle E^{gw}_{solv.}\rangle - \langle E^{go}_{solv.}\rangle$, $\Delta G_{cw} = \langle E^{gw}_{solv.}\rangle - \langle E^{gc}_{solv.}\rangle$, where $\langle E^{gw}_{solv.}\rangle$, $\langle E^{go}_{solv.}\rangle$, and $\langle E^{gc}_{solv.}\rangle$ are means of the solvation terms obtained for the conformers accumulated during the last 1000 MC steps. A similar approach was applied to estimate solvation contribution into the cyclohexane-water free energy of transfer for Ac-Ala-NMe and the 25-residue α-helix of poly-L-Ala. In this last case only 100 MC steps were performed.

RESULTS AND DISCUSSION

Development of the atomic solvation parameters

The procedure used to derive ASPs is similar to those employed previously by other authors in implicit solvent simulations of globular proteins (e.g., Eisenberg and McLachlan, 1986; Ooi et al., 1987; Wesson and Eisenberg, 1992; Schiffer et al., 1993; Cummings et al., 1995). The main difference is a number and definition of the ASP-atom types. Also, only few sets of ASP-like parameters are used today for simulations of full-atom models of peptides in nonpolar media (e.g., Ducarme et al., 1998) and, moreover, they are incorporated into force fields different from that applied in this study. Therefore, the proposed solvation model has a number of features that distinguish it from the others. This also implies that the new ASPs should be rigorously tested. To find an optimal (minimal) number of parameters and, hence, to avoid overfitting of the data, the linear system (2) was solved for various numbers (M) of the ASP types. Choice of the optimal M was based on the analysis of a discrepancy, δ, and a square of the multiple correlation coefficient, $R^2(M)$, between experimental ($\Delta G_{exp.}$) and calculated ($\Delta G_{calc.}$) energies of transfer. Analysis of δ and $R^2(M)$ for $ASP_{gc,gw,ow}$ (Fig. 1) shows that the choice of eight ASP-types is optimal: further increasing M does not lead to increasing $R^2(M)$ and decreasing δ, whereas employment of smaller M reveals decreasing $R^2(M)$ and growth of δ, respectively. In addition, the choice M = 8 is corroborated by inspection of hydrophobicity constants of the ASP-atom types. Such constants derived from the analysis of octanol/water partition coefficients are widely used to assess polarity properties of molecules via molecular hydrophobicity potential (MHP) calculations (e.g., Efremov and Alix, 1993). For the eight ASP types proposed here, the values of the MHP-hydrophobicity constants (Ghose and Crippen, 1986) are rather different and, therefore, it is reasonable to separate these ASP types in parametrization.

An important feature of our solvation model is a treatment of charged atoms. We assigned the same ASP-type ($O^{-1/2}$) for all charged oxygens because in proteins they most often belong to COO^- groups. Charged nitrogens are presented both in shared pairs with effective atomic charge q = +0.5 (like atoms Nζ1, Nζ2 in Arg) and in an isolated state with q = +1 (Nζ in Lys). We found that employment of two ASP types ($N^{+1/2}$ and N^{+1}) instead of one ($N^{+1/2,+1}$) does not lead to decreasing error in solving the linear system (2) (data not shown). Therefore, we attributed all the charged nitrogens to the same ASP type. A similar criterion was used to attribute nitrogens and carbons in the heteroaro-

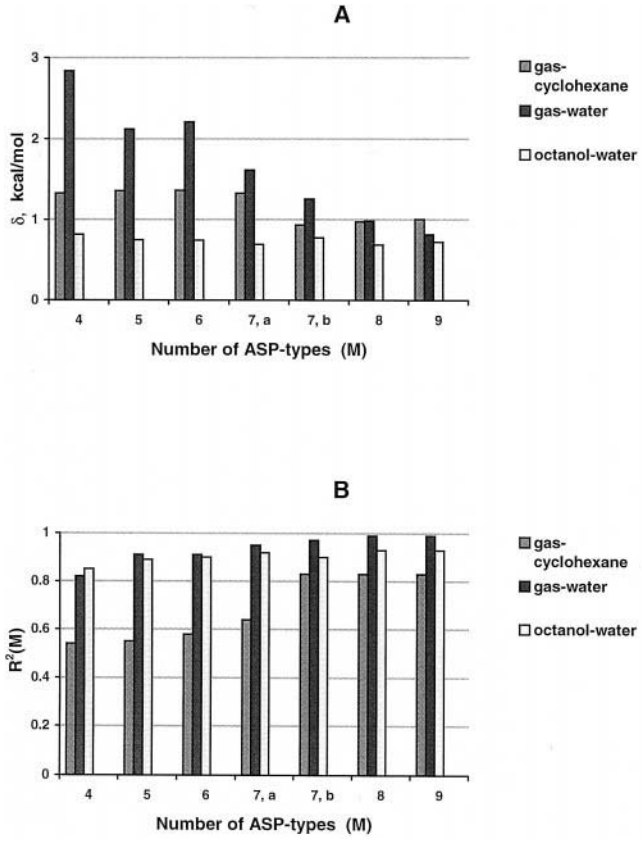

FIGURE 1 Comparison of experimental values of ΔG [taken from Sharp et al. (1991)] for free energy of transfer of amino acid residues with the values of ΔG calculated using three sets of atomic solvation parameters (ASP) for various solvents and different numbers (M) of ASP types. δ, standard deviation calculated according to the formula

$$\delta = \sqrt{\frac{1}{N-M}\sum_{i=1}^{N}(\Delta G_i^{exp.} - \Delta G_i^{calc.})^2},$$

where $\Delta G_i^{exp.}$ and $\Delta G_i^{calc.}$ are experimental and calculated energies of transfer for residue of type i, respectively. N is a number of residue types. $R^2(M)$, square of the multiple correlation coefficient between $\Delta G^{exp.}$ and $\Delta G^{calc.}$. See text for definition of ASPs at different M.

matic ring of His to specific ASP-types (see Method of Calculation).

ASPs derived for various systems, along with corresponding standard deviations obtained upon solving the overdetermined system (2) of linear equations by the SVD algorithm, are shown in Table 1. The plots of experimental ($\Delta G_{exp.}$) energies of transfer and those calculated using the sets ASP_{gc}, ASP_{gw}, and ASP_{ow} ($\Delta G_{calc.}$) are shown in Fig. 2. Corresponding slopes of the least-squares lines (also shown in Fig. 2), are 0.75 ± 0.14, 0.98 ± 0.04, and 0.90 ± 0.13, respectively. The parameters obtained might be divided into three subsets: parameters imitating transfer from gas or protein interior (modeled by octanol) to polar (i) (ASP_{gw}, ASP_{ow}) or nonpolar (ii) environments (ASP_{gc}, $ASP_{gc'}$, ASP_{oc}), and ASPs imitating transfer from gas to protein interior (iii) (ASP_{go}). We expect that the set (i) could be employed to model an aqueous environment, while the set (ii) mimics the membrane interior. The ASP_{ow} and ASP_{oc} (Table 1) are interpreted as follows: accessibility to water is favorable only for O and N atoms, whereas membrane exposure—for aliphatic nonpolar carbons. Analysis of the resulting ASPs confirms our choice of the number (M) of atom types. Thus, even if for the ASP_{gc} set the values attributed to the carbon atoms are somewhat close to each other and all negative, those for the ASP_{gw} set differ in their absolute values and signs (Table 1). The same is true for O and N atoms in ASP_{ow} and ASP_{gc} sets, respectively.

It is not apparent what probe radius (R_p) should be used to calculate protein surface exposed to the membrane interior. Effective radius of cyclohexane molecule is ~3.3 Å, but the local curvature radius could be smaller. As seen in Table 1, ASP_{gc} and ASP_{gc}, determined with R_p = 1.4 Å and R_p = 3.3 Å, respectively, reveal strong correlation (correlation coefficient 0.98), although their absolute values slightly differ. Results of MC simulations demonstrate very similar behavior of the peptides under study when either one or the other value of R_p for cyclohexane was employed (data not shown). This makes possible to calculate all sets of ASPs with the same probe radius. The results described below were obtained using R_p = 1.4 Å. Also, as it was demonstrated by Cummings et al. (1995) and confirmed in our studies (data not shown), the values of ASPs obtained by solving the system (Eq. 2) are not very sensitive to the set of ASAs employed. Therefore, in this work we used the ASA values for residues in extended conformation. We should note that these ASAs agree fairly well with those derived by Wesson and Eisenberg (1992) from analysis of 3D protein structures.

To summarize, the number of ASPs (M = 8) adapted here exceeds the numbers for other solvation models employed in MC and MD simulations of globular proteins; there, M varies from two (Fraternali and van Gunsteren, 1996) to seven (Ooi et al., 1987). Our choice of M is based on the following results: 1) analysis of solutions (ASPs) obtained for the system of linear equations (Eq. 2) with different values of M; 2) the atom types were selected depending on their MHP-related properties; 3) the parameters found differ greatly at least in one of the ASP-sets (see below); and 4) MC simulations in nonpolar media demonstrate larger helix-forming propensities (see below) and better convergence of MC procedure (see accompanying article) with M = 8 than with the other M values. We should also note that computational efforts almost do not depend on the number of the parameters used. Finally, the only criterion of validity for each solvation model lies in its testing against experimental data, and below we will consider this in more detail.

Testing the parameters

Although the main interest we pursued in this work was development and validation of a solvent model for a membrane-like environment (set ASP_{gc}), ASPs imitating aque-

TABLE 1 Atomic solvation parameters (ASP), $\Delta\sigma$ (cal/(mol × Å^2)), derived by fitting of various sets of experimental free energies of transfer

ASP	Hydrophobic core of a membrane			Protein interior	Aqueous solution	
	gc	gc′	oc*	go#	gw	ow
$\Delta\sigma(C_{aliph})$	−11 ± 2	−5 ± 1	−5 ± 4	−6 ± 3	20 ± 2	26 ± 2
$\Delta\sigma(C_{arom})$	−26 ± 4	−14 ± 2	4 ± 6	−30 ± 5	−1 ± 4	29 ± 3
$\Delta\sigma(C_{heter})$	−26 ± 6	−10 ± 3	8 ± 9	−34 ± 7	−22 ± 6	12 ± 4
$\Delta\sigma(O)$	3 ± 15	0 ± 8	71 ± 24	−68 ± 19	−83 ± 15	−15 ± 11
$\Delta\sigma(N)$	−59 ± 19	−26 ± 10	73 ± 30	−132 ± 23	−140 ± 19	−8 ± 13
$\Delta\sigma(S)$	−2 ± 14	−3 ± 7	7 ± 22	−9 ± 17	32 ± 14	41 ± 10
$\Delta\sigma(O^{-1/2})$	−20 ± 8	−9 ± 4	94 ± 13	−114 ± 10	−128 ± 8	−14 ± 6
$\Delta\sigma(N^{+1/2,+1})$	−22 ± 12	−14 ± 5	156 ± 18	−178 ± 14	−198 ± 12	−20 ± 8

Abbreviations used for the sets of ASPs: gc, gc′, parameters for the gas-cyclohexane transfer determined with probe radii of 1.4 and 3.3 Å, respectively; oc, octanol-cyclohexane; go, gas-octanol; gw, gas-water; ow, octanol-water. Experimental values for amino acid side-chain analogs (for gc, gc′, and gw sets) and N-acetyl amides of amino acids (for ow set) corrected by Sharp et al. (1991) were used.

*The difference between ASP_{gc} and ASP_{go}.

#The difference between ASP_{gw} and ASP_{ow}.

ous (ASP_{gw}) and weak polar (octanol, ASP_{ow}) solvents were also tested. This was done because analysis of the results obtained in simulations with different solvent models as well as in vacuo provides an additional insight into the specific role of environment in determining structural and energetic properties of peptides in membranes. In addition, polar ASPs will be further included in the hetero-phase model of the bilayer, which is under development now, whereas octanol is often used to approximate an environment inside a protein globulum. To inspect whether our ASPs permit reasonable estimation of the energetics of solvation in the three types of environment mentioned above, we applied the following tests for systems that were not employed in the development of the parameters.

Calculation of the free energies of transfer from the acyl chain region of the bilayer to water for Ac-Gln-X-Ile-NMe peptides, where X = Gly, Trp, Ala

This system (system i) was chosen because it provides an intriguing opportunity to compare our solvation parameters imitating the aqueous solvent and hydrophobic membrane core with recent experimental data of Thorgeirsson et al. (1996). These authors directly determined free energies of transfer from phospholipid bilayers to water ($\Delta G_{bilayer}$) for side chains of different residues introduced individually at a guest site in a 25-residue peptide derived from yeast cytochrome c oxidase. It is important that the guest site location with respect to the bilayer was controlled in the experiments. Thus, the hydrophobic guest residues were shown to be immersed in the acyl chain region of the membrane. To validate our parameters for the nonpolar core of the bilayer and for water, we roughly estimated the values of $\Delta G_{bilayer}$ and compared them with those obtained experimentally. The calculations were done for the peptides Ac-Gln-X-Ile-NMe because Gln and Ile were the neighbors of the guest site (X) in the experimental work (Thorgeisson et al., 1996). Two hydrophobic residues with large (Trp) and small (Ala) side chains were selected for this test. (According to the experimental data, the immersion depth of the polar quest sites was rather lower and, most probably, reflects their location in the headgroup region of the bilayer.) The experimental and calculated values of $\Delta G_{bilayer}$ (relative to glycine), respectively, are Trp, 2.46 ± 0.15 and 2.82 ± 0.94; Ala, 0.64 ± 0.15 and 1.75 ± 1.03 kcal/mol.

Calculation of the free energy of cyclohexane-water transfer for 25-residue α-helix of poly-Ala and for the side chain of Leu

The availability of recent theoretical data (Ben-Tal et al., 1996) on the solvation term contribution into the free energy of hydrocarbon-water transfer for 25-mer polyalanine α-helix motivated the choice of system ii. In the work of Ben-Tal et al. (1996), the hydrophobic cost of the helix insertion into the liquid alkane phase (imitating the membrane), was estimated to be ~−36 kcal/mol. In our simulations the mean value of the solvation energy ($\langle E_{solv.}\rangle$) is −30.4 ± 1.4 kcal/mol. Moreover, calculated independently (for Ac-Leu-NMe relative to Ac-Gly-NMe) free energy reduction associated with partitioning of the leucine side chain into a nonpolar membrane core is ≅3.3 kcal/mol, whereas the experimental value is ~3 kcal/mol (Wimley and White, 1996).

Calculation of the free energy of octanol-water transfer for Ac-Trp-Leu$_m$ (m = 1, 2, 3, 4) peptides

ASPs based on the octanol-water energies of transfer are widely used in MD and MC simulations of globular proteins (e.g., Eisenberg and McLachlan, 1986; Schiffer et al., 1993; Stouten et al., 1993; von Freyberg et al., 1993; Cummings et al., 1995; Juffer et al., 1995). To inspect our set ASP_{go} for its ability to reproduce known experimental data, we introduced test system iii. Experimental free energies of transfer of Ac-Trp-Leu_m (m = 1, 2, 3, 4) peptides from octanol to

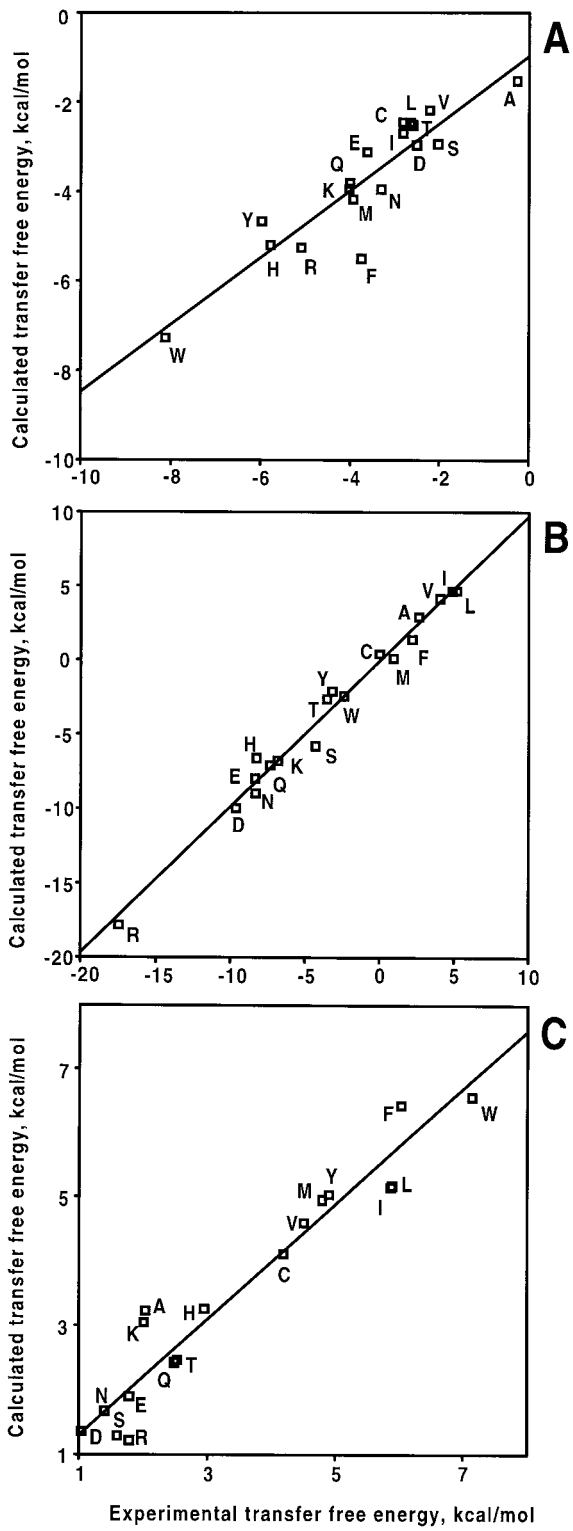

FIGURE 2 Comparison of experimental values $\Delta G_{exp.}$ for free energy of transfer for N-acetyl amino acid amides with the values of $\Delta G_{calc.}$ calculated from Eq. 2 with the following sets of atomic solvation parameters (ASP): A, ASP_{gc}; B, ASP_{gw}; C, ASP_{ow}. The least-squares are also shown.

water (ΔG_{ow}) were reported by Wimley and White (1996). As seen in Table 2, the estimations of $\langle E_{solv.} \rangle$ agree fairly well with the corresponding experimental values. Probably,

TABLE 2 Experimental and calculated free energies of transfer of Ac-Trp-Leu_m peptides from octanol to water

m	ΔG_{ow}, Experiment*	ΔG_{ow}, calculation#
1	-0.92 ± 0.05	-0.97 ± 0.30
2	0.22 ± 0.04	0.24 ± 0.32
3	1.19 ± 0.01	1.17 ± 0.34
4	2.29 ± 0.01	1.78 ± 0.35

*Data taken from Wimley and White (1996).
#This work.

the somewhat larger discrepancy obtained for $m = 4$ could be explained by partial aggregation of the peptide in aqueous phase (Wimley and White, 1996) as well as formation of secondary structure in the course of MC simulations. In addition, relatively large errors in determination of ΔG obtained for all three test systems are caused by the fact that these values are calculated as a difference of two or more terms ($\langle E_{solv.} \rangle$) known with uncertainties (standard deviation) ~4–5%.

Estimation of the free energy change associated with protonating of the C terminus of Ac-Trp-Leu_m (m = 1, 2) peptides in water.

The free energy cost of protonation of the C-terminal COO^- group in two peptides, Ac-Trp-Leu and Ac-Trp-Leu-Leu, has been estimated using the ASP_{gw} set (test system iv). The solvation contribution to the free energy has been calculated as the difference of $\langle E_{solv.} \rangle$ values obtained in MC simulations of protonated and deprotonated peptides. Resulting values are 2.91 ± 0.16 and 3.02 ± 0.17 kcal/mol, respectively, while the experimental one is 2.68 ± 0.10 kcal/mol (Wimley and White, 1996).

We should indicate that in the tests described above, all ASP types, except N^+, were employed. The absence of class N^+ in the systems considered here is explained by the lack of reliable experimental data on free energies of transfer for small peptides containing charged nitrogen atoms. At the same time, simulations for TM peptides presented in the accompanying paper demonstrate validity of ASPs for N^+. In addition, missing this ASP type in the test systems does not influence the choice of optimal M, because N^+ atoms are presented in all sets of ASPs obtained with different values of M.

Thus, to the first approximation, the ASP-based estimations of $E_{solv.}$ permit assessment of (in accord with the experimental data) principal trends in energetics of solvation for peptides in environments of different polarity. However, several important aspects should be discussed. 1) How consistent is the combined force field with added $E_{solv.}$?; 2) what is the role of long-range solute-solvent electrostatic interactions?; 3) how justified are estimations of ΔG based on consideration of $E_{solv.}$ only, without taking into account other energy terms?; and 4) what is the quality of MC sampling upon calculations of ΔG?

According to its origin (fitting to experimental ΔG), $E_{solv.}$ is well-suited to reproduce free energies of transfer between different solvents. However, the physical meaning of ASPs and their weight against force field parameters in vacuum are not exactly clear. The pseudo-energy term $E_{solv.}$ was simply added to the ECEPP/2 (vacuum) potential to give the total energy of a system (Eq. 3), and the ECEPP/2 energy terms were not used to derive ASPs from experimental data. Therefore, validity of such a hybrid force field is not generally apparent. First, it might lead to inconsistency of the energy function because its different terms were developed based on different sets of experimental data. Second, in the result of such a procedure, several interactions might be accounted twice (e.g., electrostatic effects appearing in $E_{ECEPP/2}$ are implicitly included in $E_{solv.}$). To avoid (at least partially) double accounting of electrostatic effects, the long-range electrostatic interactions were significantly dumped by using a distance-dependent dielectric permeability, although short-range electrostatic interactions contributing to the H-bonding term were explicitly included. To inspect whether our solvent model is sensitive to ε, we have performed a short MC-conformational search for 10-residue poly-Leu with ASP_{gc} and $\varepsilon = 4 \times r$ and $\varepsilon = 2$ (this last value is often used to model a hydrophobic membrane environment). Starting from the same random coil conformation, the lowest-energy structures found after 1500 MC steps contained six and four residues in α-helix, respectively (as it will be shown below, in a case of 20-residue poly-Leu more sophisticated search reveals all-helical conformation for $\varepsilon = 4 \times r$). This shows that dumping of the electrostatic contribution (with solvation and H-bonding terms switched on) promotes α-helix formation in nonpolar media. That is why the other results described here were obtained with $\varepsilon = 4 \times r$.

The problems just discussed are common for all empirical force fields. In such a situation the criterion of validity of the combined force field is accordance between calculated and experimental data. Sometimes, initial ASPs derived from the experimentally measured free energies of transfer are subjected to refinement to reproduce the results of simulations with explicit solvent (Schiffer et al., 1993; Fraternali and van Gunsteren, 1996). Here, in Eq. 3 we used weighting factor 1 for $E_{solv.}$ just because the results obtained for small peptides (see above) as well as for TM peptides (described in the accompanying paper) reveal reasonable balance between $E_{solv.}$ and $E_{ECEPP/2}$, thus driving the conformational search in a "right direction." For the small-size test systems considered here, one of the important reasons for this lies in the absence of self-association and secondary structure creation during the simulations and, hence, the energetics of such processes (e.g., helix formation) did not contribute to the free energy of transfer. Analysis of various ECEPP/2 terms for the same peptide in different solvents shows that they are comparable (data not shown), and the major effect on ΔG was caused by $E_{solv.}$. But we should stress that the general assumption that the intramolecular contribution to E_{total} is solvent-independent is not valid. Thus, for larger peptides the energy associated with the formation of a secondary structure could not be neglected when considering partitioning of peptides between different environments. That is why testing of ASPs for systems i ÷ iv only partially (in the simplest cases) addresses problems of water/bilayer partitioning and does not consider energetics of protein adsorption on the bilayer and membrane insertion.

As discussed above (see Method of Calculation) the procedure used to estimate ΔG is quite approximative because instead of Boltzmann ensemble we used a sample of only ~300 different states that correspond to local minima on the potential energy surface (acceptance rate in these MC runs was ~30%). However, for such small systems even this restricted conformational search led to reasonable results and, therefore, the approximations made seem to be justified. Obviously, for larger systems calculation of ΔG calls for rather elaborate techniques, but detailed analysis of free energy of the system peptide plus membrane is not the subject of the present work.

Another question that should be answered is how can simulations with ASPs representing a bulk solvent be used to mimic heterogeneity of real membranes? In connection with this we need to outline that our objective here is to check how different ASPs influence conformational, H-bonding, etc. properties of peptides that are assumed to traverse a bilayer. In such a case, most of the peptide is immersed in the nonpolar core of the membrane and, according to our idea, could be properly described by the ASP_{gc} set. This determined our choice of test systems i and ii: residues in the acyl chain region of the bilayer. As we will show in the accompanying paper, such approximation provides fairly good results for real TM peptides; generally, only one or two terminal residues demonstrate properties that might differ from those observed in the experiment.

MC simulations of homopolypeptides: poly-Leu, poly-Val, poly-Ile, and poly-Gly

Before testing ASPs on real TM peptides, we have to mention one more aspect of the problem that should be tractable in a case of ASPs mimicking the membrane environment. It is well known that bilayer significantly promotes helix formation (Deber and Li, 1995; Deber and Goto, 1996; Liu et al., 1996). A pictorial example is provided by C_β-branching residues as Val and Ile: they are often found in membrane-spanning segments in α-helical conformation, but in aqueous solution reveal helix-destabilizing properties (Padmanabhan et al., 1990). Therefore, solvent models imitating a membrane should be also tested for their ability to favor helix formation for certain residues. On the contrary, α-helical propensities for the residues should be significantly smaller in simulations with water-mimicking ASPs. To address these questions we employed our sets of ASPs to study conformational properties of Val, Ile, Leu, and Gly residues in different environments. Leu

and Gly in water and in the bilayer demonstrate high and small helix-forming propensities, respectively (Blaber et al., 1993), and they were chosen as reference residues.

The peptides were taken in random conformations and subjected to a multi-step conformational search in vacuo as well as with two sets of ASPs mimicking nonpolar solvent and water. In the result, conformational space of the peptides was intensively sampled, and the energy-minimized conformers accepted by the Metropolis criterion were selected for subsequent analysis. Quality of the sampling was checked by inspection of all trial values of dihedral angles φ and ψ for residues in the homopolypeptides. It was found that these values are almost uniformly distributed on the φ/ψ map (data not shown). Acceptance rates in the MC procedure varied between 14 and 35%, depending on the simulation conditions, like the number of dihedrals sampled on each MC step, temperature schedule, etc. The values of φ and ψ in the accepted conformations were found in the regions corresponding to coil and right-handed α-helix conformations on Ramachandran's plot. Only very few accepted conformers (maximum two for each polypeptide) were found to contain short (2–3 residues) fragments of extended structure (assigned to "E" by the DSSP program), and their energies were rather far from the lowest energy minima (data not shown). Therefore, in further discussion we will focus on analysis of environment-dependent distribution of the α-helix in these peptides.

For accepted conformers of each homopolypeptide, a number of residues in α-helical conformation versus total energy of the system is shown in Fig. 3. Because all these structures were minimized during the search, each point on these plots corresponds to individual local minima on the potential energy hyper-surface characteristic for a given peptide in a particular solvent. The lowest-energy conformers are presented in Fig. 4, and some of their characteristics are listed in Table 3. Analysis of these data permits the following conclusions:

1. In membrane-like surroundings, the lowest-energy conformations for all the peptides (except poly-Gly) have the largest α-helical content and largest values of ASA;
2. In vacuum, numerous conformers with significant helical content were found for Leu and Ile, and to a smaller degree for Val and Gly. The lowest-energy conformations in vacuum do not correspond to maximal helicity of the peptides;
3. In water, no α-helical fragments were found for poly-Ile. For poly-Leu and poly-Val a population of conformers with one helix turn (4 residues) was found. One of these structures for poly-Leu corresponds to the lowest-energy minimum, while for poly-Val it does not;
4. For poly-Gly, numerous conformers with up to nine residues in the α-helix were observed in water, but their energies were rather higher than the lowest-energy minimum found for unordered structures;
5. For hydrophobic residues, the energy gaps between the lowest-energy conformers with and without α-helix are rather higher in nonpolar solvent than in vacuum: for poly-Leu, Val, and Ile they are 78.05, 51.07, 53.31, and 33.62, 6.77, and 28.39 kcal/mol, respectively. (For poly-Gly the corresponding values are very close: 7.89 and 5.86 kcal/mol.) Moreover, in a nonpolar environment these energy gaps separate states with maximal and zero helical content, while in vacuum the most energetically stable conformers have no maximal helicity;
6. In nonpolar solvent, the states with maximal helicity ($N_\alpha^{\max.}$) are overlapped with the other states having $N_\alpha < N_\alpha^{\max.}$ (Fig. 3).

To estimate how the number of atom types (M) influences the results in nonpolar media, we performed a restricted MC conformational search for 10-residue poly-Leu with ASPs derived for $M = 4$, 5, and 8. Starting from identical coil structures, we found 0, 4, and 6 residues in α-helix in the corresponding lowest-energy conformers. Therefore, at $M = 8$, the peptide's conformational space is characterized by a higher population of states (local minima) corresponding to the α-helix than in the case of $M = 4$ and 5. This provides an additional argument (see Development of the Atomic Solvation Parameters) supporting our choice of $M = 8$.

To summarize, we outline that a nonpolar solvent (and to a lesser degree, a vacuum) promotes α-helix formation in all the peptides. This is especially pronounced for poly-Leu. At the same time, a conformational landscape for the peptides is different for solvent mimicking the hydrophobic core of a membrane, and in a vacuum. That is why one should take care when simulating TM segments of proteins in vacuo. This last statement will be also illustrated in the accompanying paper by simulations of membrane-bound peptides in different solvents. In aqueous solution a stable α-helix was observed only for poly-Leu, although it contained only four residues. This is consistent with the fact that water generally destabilizes the helical structure by competing for formation of H-bonds within the peptide backbone (Tirado-Rives and Jorgensen, 1991; DeLoof et al., 1992). The lowest-energy conformers obtained with ASP_{gw} demonstrate rather smaller values of ASA (Table 3) than in nonpolar media, thus confirming that the peptides shield their hydrophobic side chains from aqueous surroundings. As it was reasonable to expect, conformational properties of Gly in all tested environments are rather different from those for hydrophobic residues: despite partial helix formation in nonpolar media, they tend to reduce surface area accessible to solvent via compact packing (Fig. 4 *N*). On the contrary, the lowest-energy conformers of poly-Leu, Val, and Ile adapt an α-helical conformation without kinks (with maximal ASAs) which can traverse the bilayer (Fig. 4, *F* and *J*).

Using CD spectroscopy, Deber and co-authors (Deber and Li, 1995; Deber and Goto, 1996; Liu et al., 1996) measured helicity of various peptides (including the Gly-containing ones) in membrane-mimetic media and demonstrated significant membrane-promoting helix formation for them. The largest helical propensities were observed for Ile,

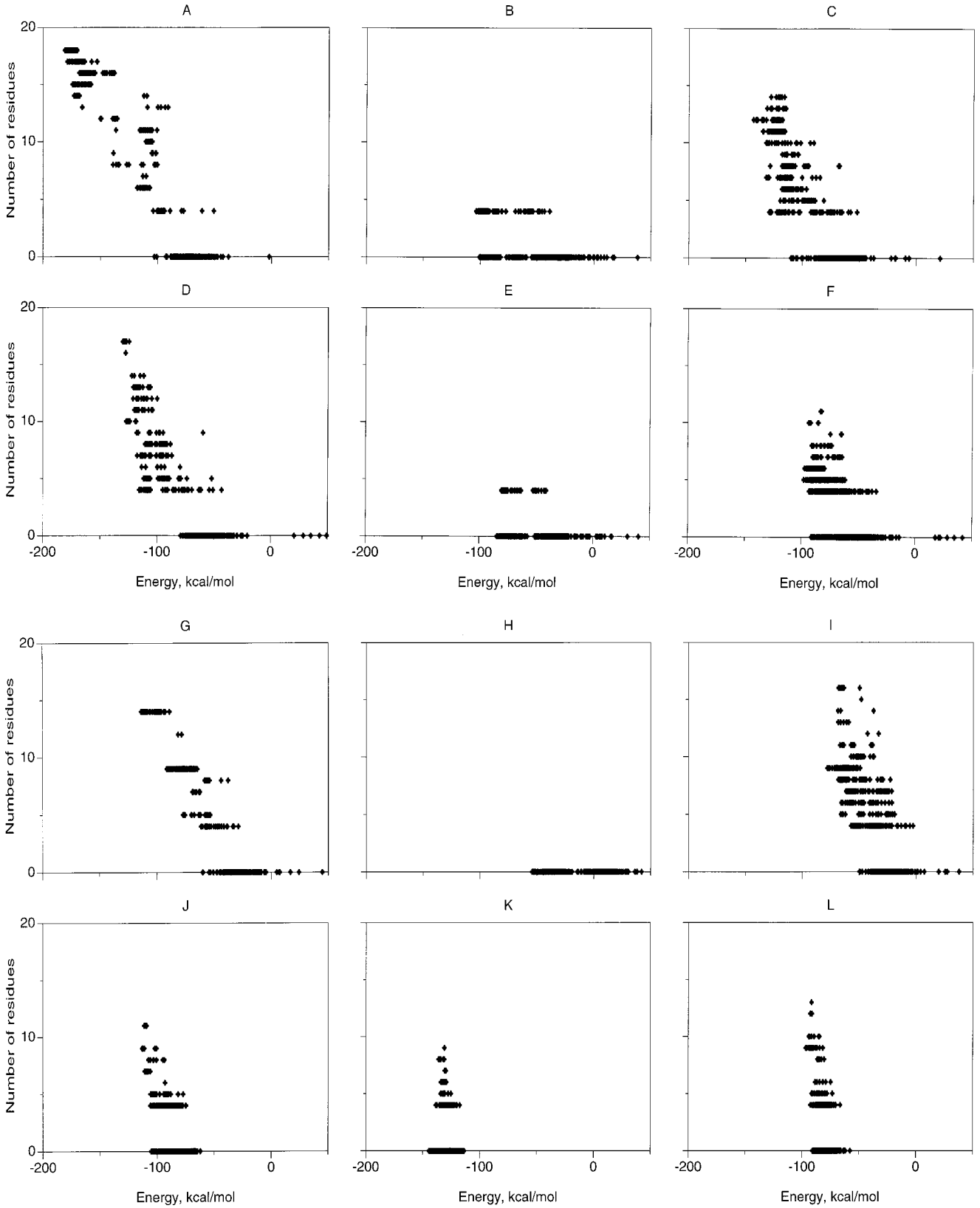

FIGURE 3 α-Helical content of homopolypeptides versus total energy for accepted conformers found in the result of nonrestrained Monte Carlo conformational search in different environments. (*A–C*) Poly-Leu in nonpolar solvent, water, and in vacuo, respectively; (*D–F*) the same for poly-Val; (*G–I*) the same for poly-Ile; (*J–L*) the same for poly-Gly. Initial structures were taken in random conformations. Each point on the plots represents energy-minimized structure.

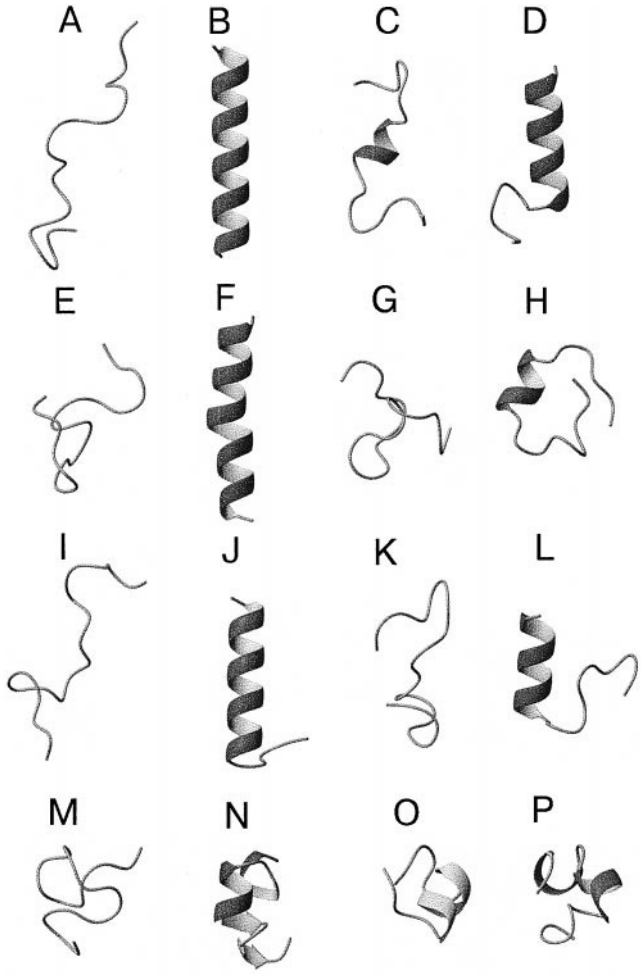

FIGURE 4 Ribbon representation of initial and lowest-energy conformers of poly-Leu, poly-Val, poly-Ile, and poly-Gly obtained in the result of nonrestrained Monte Carlo simulations in different environments. Poly-Leu: (*A*) initial structure (random); (*B–D*) lowest-energy conformers obtained with ASP_{gc}, ASP_{gw}, and in vacuo, respectively. (*E–H*) the same for poly-Val; (*I–L*) the same for poly-Ile; (*M–P*) the same for poly-Gly.

Leu, and Val. A conclusion was made that although C_β-branched side chains of Val and Ile may sterically interfere with the carbonyl oxygen in the preceding turn of the helix, and hence destabilize α-helical conformation (O'Neil and DeGrado, 1990), in nonpolar solvent this effect may well be balanced by favorable interactions of hydrophobic side chains with solvent. In the result, in micelles and vesicles Val- and Ile-containing peptides adapt conformations with high helical content. Therefore, the results of our simulations are in good agreement with experimental observations and, moreover, provide additional insight into details of the energetic landscape of the peptides in different environments.

CONCLUSIONS

This study presents results on development and testing of an implicit solvation model for proteins and peptides. Special care was taken to select an optimal number of parameters of the model. In the result, eight atom types were adapted and corresponding different sets of ASPs were derived. This permits effective representation of protein-solvent interactions in the acyl chain region of the membrane, aqueous solution, and weak polar media (octanol). The model was tested by estimation of bilayer-water, hydrocarbon-water, and octanol-water free energies of transfer for a series of short peptides, and comparison of the results with known experimental data. Reasonable overall agreement between the measured and calculated data was reached. A conclusion was made that the approach, being rather less CPU-demanding than explicit solvent simulations, correctly accounts for effects of environment and provides feasible balance between solvation and other energy terms in the potential energy function.

Furthermore, the solvation model was employed in nonrestrained MC simulations to explore conformational space of four homopolypeptides—poly-Leu, poly-Val, poly-Ile, and poly-Gly—in membrane-mimicking media, water, and in vacuo. In accordance with experimental observations, significant membrane-promoted α-helix formation was observed. In nonpolar media the lowest-energy conformers of poly-Leu, Val, and Ile reveal highest α-helical content and exposure to solvent. On the contrary, aqueous solution was shown to destabilize the helical structure (except poly-Leu, where stable helical segments were found). Although the energy landscape for the peptides in vacuum contains numerous deep local minima corresponding to partially helical structures, the vacuum simulations do not favor α-helix formation as efficiently as with the membrane-mimicking ASPs. Therefore, simulations in vacuo can miss the important details in structure and energetics of membrane-bound peptides.

TABLE 3 Helicity (N_α) and accessible surface area (ASA) of the lowest-energy conformers of homopolypeptides obtained in the result of Monte Carlo simulations in vacuo and with different sets of atomic solvation parameters (ASP)

Peptide	Membrane-mimicking parameters, ASP_{gc}	Water-mimicking parameters, ASP_{gw}	Vacuum
poly-Val			
N_α*	17	0	5 ÷ 6
Total ASA,# Å^2	1932 ± 7	1725 ± 2	1776 ± 24
poly-Ile			
N-helix	14	0	9
Total ASA, Å^2	2173 ± 2	2068 ± 39	2007 ± 30
poly-Leu			
N_α	18		12
Total ASA, Å^2	2288 ± 2	2172 ± 4	2250 ± 8
poly-Gly			
N_α	9	0	12
Total ASA, Å^2	1106 ± 4	1078 ± 6	1068 ± 9

*Number of residues in α-helix as assigned by the DSSP program (Kabsch and Sander, 1983) for the set of 10 lowest-energy conformers. Two terminal residues were not counted.
#As assigned by the DSSP program for the set of 10 lowest-energy conformers.

We stress that the ASP-based solvation model presented here will be adequate mainly for peptides immersed in the acyl chain region of the membrane, e.g., TM segments in proteins. Another limitation of the approach is the absence of the peptide's influence on the structure of the bilayer. Also, the model does not address problems related to peptide partitioning on the water-bilayer interface and membrane insertion. To account for such effects, a more advanced three-phase membrane model based on combined employment of parameters for water and hydrocarbon is now under development.

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