Molecular Dynamics Study of Substance P Peptides in a Biphasic Membrane Mimic

Troy Wymore and Tuck C. Wong
Department of Chemistry, University of Missouri, Columbia, Missouri 65211 USA

ABSTRACT Two neuropeptides, substance P (SP) and SP-tyrosine-8 (SP-Y8), have been studied by molecular dynamics (MD) simulation in a TIP3P water/CCl_4 biphasic solvent system as a mimic for the water-membrane system. Initially, distance restraints derived from NMR nuclear Overhauser enhancements (NOE) were incorporated in the restrained MD (RMD) in the equilibration stage of the simulation. The starting orientation/position of the peptides for the MD simulation was either parallel to the water/CCl_4 interface or in a perpendicular/insertion mode. In both cases the peptides equilibrated and adopted a near-parallel orientation within ~250 ps. After equilibration, the conformation and orientation of the peptides, the solvation of both the backbone and the side chain of the residues, hydrogen bonding, and the dynamics of the peptides were analyzed from trajectories obtained in the RMD or the subsequent free MD (where the NOE restraints were removed). These analyses showed that the peptide backbone of nearly all residues are either solvated by water or are hydrogen-bonded. This is seen to be an important factor against the insertion mode of interaction. Most of the interactions with the hydrophobic phase come from the hydrophobic interactions of the side chains of Pro-4, Phe-7, Phe-8, Leu-10, and Met-11 for SP, and Phe-7, Leu-10, Met-11 and, to a lesser extent, Tyr-8 in SP-Y8. Concerted conformational transitions took place in the time frame of hundreds of picoseconds. The concertedness of the transition was due to the tendency of the peptide to maintain the necessary secondary structure to position the peptide properly with respect to the water/CCl_4 interface.

INTRODUCTION

Peptide-lipid membrane interactions represent an important field in biophysics, biochemistry, and pharmacology (White and Wimley, 1994; Schwyzer, 1992). These interactions are central to the insertion and folding of membrane proteins, the action of antibiotic peptides, and the rupturing of membranes by toxins. Understanding the accumulation, conformation, and orientation of peptides on lipid membranes is also a key to the molecular mechanism of receptor selection (Sargent and Schwyzer, 1986; Schwyzer, 1995). Though the conformation of such peptides can be studied by a variety of spectroscopic techniques, the precise orientation of the entire peptide backbone is less certain and the side chain locations are usually only probed for those that contain either a chromophore or a spin label (Merz and Roux, 1996). An accurate three-dimensional description of the peptide-membrane complex requires the incorporation of many experimental techniques that can be complicated by the requirement of different conditions for which each technique is applicable. Recent advances in solid state NMR have shown that detailed orientational/positional information can be obtained for peptide/bilayer systems (Opella, 1997). Our approach to the study of peptide-membrane complexes is through the combination of multidimensional NMR and molecular dynamics (MD) simulation, which is increasingly being used to study complex biopolymer systems (Philippopoulos et al., 1997; Bremi et al., 1997; Peng et al., 1996). NMR can be used to determine structure as well as dynamics through relaxation measurements (Palmer et al., 1996), while MD simulations can reproduce experimental observables and provide atomic level details of the structure and dynamics of biopolymer systems (Brooks et al., 1988).

Estimates of how peptides may orient at the membrane interface have been made by Schwyzer (1995), who considered four parameters in his theory: hydrophobic association, amphiphilic moment, electric dipole moment, and net charge. Schwyzer predicted many peptides that possessed primary amphiphilic moments to be oriented in a perpendicular fashion at the interface. A neutron diffraction study (Jacobs and White, 1989) found A-X-A-*O-tert*-butyl peptides (X = Leu, Phe, Trp) to be oriented parallel to the interface. An NMR study of A-F-A-*O-tert*-butyl peptide came to the same conclusion (Brown and Huestis, 1993). The peptides magainin and alamethicin adopt the insertion mode only after a significant fraction has been accumulated on the membrane surface (Ludtke et al., 1996; He et al., 1996). Two questions may be asked for peptides too short to traverse the lipid membrane: 1) does the primary amphiphilic moment of a peptide as defined by Schwyzer play a significant role in determining how the peptide interacts with a lipid membrane?, and 2) what is the most probable position of peptide residues on the membrane surface; specifically is it likely that the whole residue (side chain plus the backbone) or just the side chains that are inserted into the lipid region?

The use of a biphasic cell as a membrane mimetic for the study of peptide conformation and orientation has been

Received for publication 12 June 1998 and in final form 24 November 1998.

Address reprint requests to Dr. Tuck C. Wong, Department of Chemistry, University of Missouri, 123 Chemistry Building, Columbia, MO 65211. Tel.: 573-882-7725; Fax: 573-882-2754; E-mail: chem1060@showme.missouri.edu.

0006-3495/99/03/1199/14 $2.00

described by Guba and Kessler (1994). Their membrane mimetic used water and a united atom model for carbon tetrachloride for the simulation cell in the study of the bradykinin antagonist Hoe 140 (Guba et al., 1994) and the peptide hormone lipo-CCK (Moroder et al., 1993). Pellegrini and Mierke (1997) used the same model to study threonine^6-bradykinin and found that NMR data supported two conformations while the peptide oriented parallel to the interface. The same approach has also been applied to the study of a polyether antibiotic, monensin (Mercurio et al., 1997). The transfer of methane across the water-carbon tetrachloride interface has been simulated using a polarizable potential model (Chang and Dang, 1996). Other water/hydrophobic biphasic systems have also been studied by MD. For example, the anesthetic 1,1,2-trifluroethane was simulated in a water-hexane interfacial system (Pohorille et al., 1996). Although little is known experimentally about peptides at these interfaces at the present time, the biphasic solvent model does possess the two essential phases to approximate the hydrophobic/hydrophilic interface found in micelles and lipid bilayers, and results from work mentioned above are promising. The information gained from simulations in the biphasic cell are more accurate than those done in vacuum (see Discussion). This membrane mimetic significantly reduces the number of atoms required and the computational expense of equilibration when compared to simulations in micellar or lipid bilayer media. The membrane interface has also been approximated by a continuum model that contains two regions with different dielectric constants (Area et al., 1995). Biggin et al. (1997) used a simple bilayer model with a transbilayer voltage difference to examine possible mechanisms of the insertion of the alamethicin helix. MD studies of peptides with explicit membrane media have also been reported (Damodaran and Merz, 1995; Damodaran et al., 1995; Tobias et al., 1996; Huang and Loew, 1995; Woolf and Roux, 1996; Shen et al., 1997; Bernèche et al., 1998).

A simplistic view of the mechanism of a peptide interacting with a membrane interface is that the peptide will want to partition the hydrophobic residues in the hydrocarbon region while the hydrophilic residues remain in the aqueous phase and therefore form secondary structures in order to accomplish this most favored interaction. This picture generally tends to neglect one important aspect: the polar peptide backbone. Theoretical studies (Ben-Tal et al., 1996, 1997) reveal that the energetic cost of inserting a non-hydrogen-bonded peptide into a nonpolar region is quite high. Furthermore, if the peptide to be inserted is not of sufficient length to traverse the bilayer it must pay this thermodynamic penalty unless these peptide bonds are able to adopt a conformation that allows the peptide bonds to hydrogen-bond with nearby side chains (Ben-Tal et al., 1996). Roseman (1988) has estimated that the cost of partitioning a non-hydrogen-bonded peptide bond into a hydrocarbon phase is 5–6 kcal/mol, which is significantly larger than the free energy reduction of ~3 kcal/mol associated with partitioning of even the most hydrophobic amino acid side chain (Wimley et al., 1996). These results, both experimental and theoretical, argue against the insertion of most small peptides possessing primary amphiphilicity. Instead, small peptides will be located at the membrane surface with the hydrophobic side chains making contact with the nonpolar lipid region. To better understand the important properties of peptide-membrane systems, the factors affecting the orientation and interaction of the peptides with the membrane and to interpret aspects of NMR data for the peptides in membrane-mimicking systems obtained from our own laboratory and elsewhere, we have undertaken an MD study of substance P (SP) and its Tyr-8 analog (SP-Y8) in a biphasic cell made up of water (TIP3P model) and carbon tetrachloride. In addition, we will examine the validity of such a simplified model by comparing the results from this work to results from simulations carried out using an explicit model of a SDS micelle described in the following paper.

SP is an 11-residue neuropeptide with the sequence RPKPQQFFGLM-NH_2. SP is widely distributed in the central and peripheral nervous system and in the gastrointestinal tissue (Nicoll et al., 1980), and is involved in biological functions such as pain transmission and smooth muscle contractions (Regoli et al., 1989). SP binds to the NK1 receptor, which is coupled to G proteins (Regoli et al., 1994; Huang et al., 1994). SP has been extensively studied experimentally and therefore is a good candidate to assess our methods. The conformation of SP in micelles has been studied by two-dimensional (2D) NMR (Hicks et al., 1992; Young et al., 1994; Keire and Fletcher, 1996), Raman (Williams and Weaver, 1990) and infrared spectroscopy (Erne et al., 1986), circular dichroism and fluorescence (Woolley and Deber, 1987). SP (and SP-Y8) possesses distinct charged (the N-terminal) and hydrophobic (the C-terminal) segments, and thus possesses primary amphiphilicity as defined by Schwyzer (1992). Previous studies provided conflicting results on the orientation of SP with respect to the water/membrane interface. SP has been suggested to lie parallel to the membrane surface (Young et al., 1994; Seelig and Macdonald, 1989; Duplaa et al., 1992), whereas Schywzer (1992) suggested that SP was oriented perpendicular to the membrane surface with ~7–8 residues inserted into the hydrophobic core. It has been suggested that the hydration of the bilayers determines the orientation of similar peptides (Frey and Tamm, 1991). An MD simulation was performed for SP in a DMPC bilayer (Kothekar, 1996) starting from a perpendicular orientation with nine residues inserted as proposed by Schwyzer (Erne et al., 1986; Schwyzer, 1992). The simulation was carried out for only 260 ps, which is not of sufficient length to allow the peptide to change to the equilibrium orientation/position if the initial configuration does not correspond to the equilibrium configuration (see following paper).

SP-Y8 exhibits similar, but lower, biological activities to SP (Fisher et al., 1976). Its structure in solution and in micelles (Gao and Wong, submitted for publication) and its

partitioning in micelles as well as that of SP (Wong and Gao, 1998) have recently been studied in our laboratory.

METHODS

MD simulation details

The CHARMM program (Brooks et al., 1983) version 24b2 was used for all minimizations and simulations in the biphasic cell. The CHARMM all22 force field was used for the peptide (MacKerell et al., 1998). All MD simulations were performed in the *NVT* ensemble with application of periodic boundary conditions. The integration time step was 1 fs with bonds to hydrogen constrained to a fixed value by SHAKE (Ryckaert et al., 1977). The long-range forces were handled by using a force switch from 8 to 12 Å. This method of handling long-range forces leaves short-range forces unaltered and damps forces monotonically to zero in the interval from r_{on} to r_{off}. The energy minima and barriers introduced by force switching are considerably less pronounced than those caused by potential switching (Steinbach and Brooks, 1994). In addition, using this method for the nonbonded interactions does not require that we couple the solvents and the peptide to separate temperature baths to produce uniform temperature (Oda et al., 1996). The nonbonded list was updated every 20 fs.

Most simulations were performed on a Silicon Graphics Power Challenge computer using one R10000 processor that required 17 h of cpu time for 30 ps of simulation. Some simulations were performed on a Cray T3E computer at the Pittsburgh Supercomputing Center that required ~155 cpu hours for 100 ps of simulation. Using 32 processors on the Cray T3E allows each 100 ps section to be calculated in ~4.8 h assuming that 1 cpu hour is equal to 1 h wall time.

Construction of the biphasic cell

Separate solvent boxes of water and carbon tetrachloride were constructed by placing solvent molecules in a random orientation on a lattice. The aqueous phase consisted of 1352 water molecules and the hydrophobic phase 256 carbon tetrachloride molecules for a total of 5336 atoms. The TIP3P water model (Jorgenson et al., 1983) was used for the aqueous phase while the relevant parameters for the carbon tetrachloride model are given in Table 1 (see MacKerell et al., 1998 for further force field information). Our all-atom model of carbon tetrachloride places a negative charge on the chlorine known to be the more electronegative atom. The partial charges are taken from AIM calculations (Wiberg and Rablen, 1993). Other models place the negative charge on carbon which are taken from Mulliken population analysis. This parameter may be of small importance since the largest moment of carbon tetrachloride is the octapole and, therefore, the properties of carbon tetrachloride are probably not too dependent upon an accurate description of the electrostatics (Essex et al., 1992).

The separate solvent boxes were minimized with 1000 steps of the steepest descent minimization with a 13 Å nonbonded cutoff. The dimensions of the solvent cells were adjusted to produce the correct density—0.997 g cm^{-3} for TIP3P water and 1.594 g cm^{-3} for carbon tetrachloride—during a 10-ps dynamics simulation in which the velocities were scaled every 333 fs in 10° increments to a final value of 300 K to thermalize the separate cells and better randomize the configurations. The MD was continued at 300 K for 3 ps. The two solvent cells were then merged and minimized together with 800 steps of steepest descent. The final dimensions of the biphasic system were 43.487 × 43.487 × 43.155 $Å^3$. A 10-ps simulation was performed in which the velocities were scaled as above to a final temperature of 300 K followed by a 110-ps equilibration phase. A 300-ps simulation was also performed on the carbon tetrachloride solvent to evaluate the structure of this solvent.

TABLE 1 Lennard Jones parameters and force constants used for carbon tetrachloride model

	R_{min} (Å)	ϵ (kcal/mol)	Charge, q (a.u.)
C	1.960	0.0540	0.500
Cl	1.986	0.1608	−0.125
C-Cl (d, force)	1.767	222	
Cl-C-Cl (<, force)	109.5	77.7	

Energy (nonbonded) = $\Sigma\ \epsilon[(R_{min,i,j}/r_{ij})^{12} - 2(R_{min,i,j}/r_{ij})^{6}] + q_iq_j/(\epsilon_1 r_{ij})$, where ϵ_1 is the dielectric constant.

Simulated annealing

Distance restraints used in the simulated annealing procedure for SP were taken from Young et al. (1994) based on 2D NMR NOESY spectra obtained for SP in SDS micelles. Distance restraints for SP-Y8 were generated from NOESY spectra taken in both SDS and DPC micelles in our laboratory (Wong and Gao, 1998). Restraints during the simulated annealing were set to 200 kcal/mol Å. NOE restraints between protons were divided into three categories: strong (2.0–2.7 Å), medium (2.0–3.3 Å), and weak (2.0–4.0 Å). SP had a total of 88 restraints of which 35 were intraresidue, 31 sequential, and 22 nonsequential. SP-Y8 had a total of 106 restraints of which 48 were intraresidue, 38 sequential, and 20 nonsequential. The intraresidue restraints are of little importance since the force field usually restrains the intraresidue distances. Starting conformations were generated in an extended fashion using Sybyl 5.3 (Tripos, Inc., St. Louis, MO). No partial charges were assigned during simulated annealing. The peptides were heated by scaling their velocities to a temperature of 2000 K and cooled exponentially to 200 K over 5 ps. This process was repeated 30 times and the last 10 conformations were saved. These 10 conformations were minimized in vacuo using the CHARMM force field and a dielectric constant of 1.0, with no nonbonded cutoff. The starting structure for the molecular dynamics that had a low molecular mechanics energy with low NOE violations was chosen, though neither quantity was the lowest for all the structures considered. The final structures were all very similar and the structure used for SP closely resembled the one reported by Keire and Fletcher (1996) for SP in SDS and DPC micelles. The structure for SP-Y8 resembles that reported by Gao and Wong (submitted for publication) determined in the same two micelles.

Peptides in the biphasic cell

The peptides were placed in the equilibrated biphasic cell in two orientations with the secondary structure generated from simulated annealing based on the experimental NOE restraints. The first orientation was perpendicular to the interface to simulate an "insertion mode." The other was approximately a parallel orientation adjusted to place as many hydrophobic side chains in the carbon tetrachloride region as possible. The surrounding solvent molecules were deleted if the oxygen of TIP3P or the chlorine of carbon tetrachloride were within ~2.8 Å of any heavy atom of the peptide. A series of minimizations were performed to allow some carbon tetrachloride molecules to diffuse away from the peptide if close contacts were not too severe and the volume of the cell was expanded to maintain a more accurate density with the peptide held fixed. A steepest descent minimization was carried out for 1000 steps before molecular dynamics was started. NOE restraints were placed on the peptide as in the simulated annealing procedure. The NOE restraint force constant was reduced to 10 kcal/mol Å during this minimization and throughout the equilibration period.

The peptides were then subjected to 10 ps of heating by velocity scaling from 0 to 300 K in increments of 10. After the initial heating, velocities were scaled if the temperature was not within 10 of 300 K checked every 50 fs. During equilibration, velocity scaling was never needed after ~50 ps of dynamics. Equilibration was achieved when little change was observed for the orientational and positional properties of the peptide with respect to the interface over a 50-ps time period. The trajectory was sampled every 500 fs in which restraints were placed on the peptide. The trajectory was continued without restraints for the peptides originally placed parallel to

the interface (see Results). During this simulation period, the trajectory was sampled every 200 fs.

RESULTS

MD of biphasic cell

The carbon tetrachloride model was evaluated by examining whether it gives an accurate structure and a stable interface with water. It was found to satisfactorily reproduce the experimental radial distribution functions (Narten, 1976) for chlorine-chlorine and for carbon-carbon distances (see Fig. 1). The structure of the carbon tetrachloride solvent is similar to other simulations performed of this solvent (Tironi et al., 1996). The g(r) values are higher than those determined from experiment and some modifications to the model are perhaps desirable. During the 110-ps simulation, the interface remained stable, i.e., none of the water molecules penetrated far into the carbon tetrachloride region. A density plot of the biphasic system is shown in Fig. 2. The simulation cell clearly contains a hydrophobic/hydrophilic boundary that is crucial for studying peptides at membrane-mimicking interfaces. The final coordinates from this simulation were used for the peptide simulations.

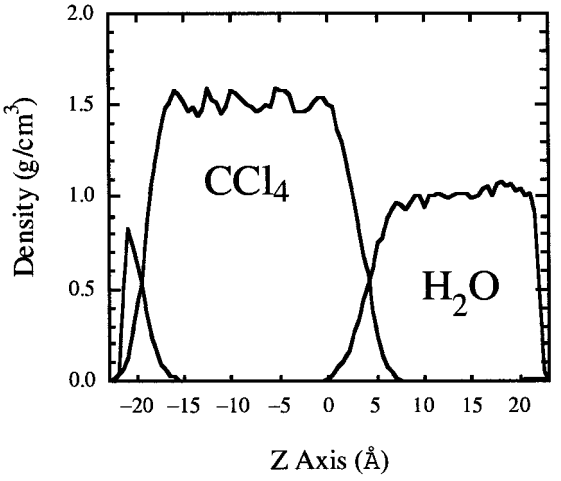

FIGURE 2 Density plot of carbon tetrachloride (*left*) and water (*right*) in the biphasic cell. The interface that appears on the left edge is due to the periodic boundary conditions.

Equilibration of peptides in biphasic cell

The equilibration of SP originally perpendicular to the interface is shown in Fig. 3. The interface between the two solvents remains throughout all simulations. The orientations that started perpendicular became parallel and those that started parallel remained in this orientation throughout the simulation. For SP originally perpendicular to the interface in the insertion mode, this equilibration process required ~500 ps, although the orientation of the peptide was essentially parallel to the interface within 300 ps. SP-Y8, which was originally perpendicular, equilibrated to a parallel orientation within 230 ps, as shown in Fig. 3. The time

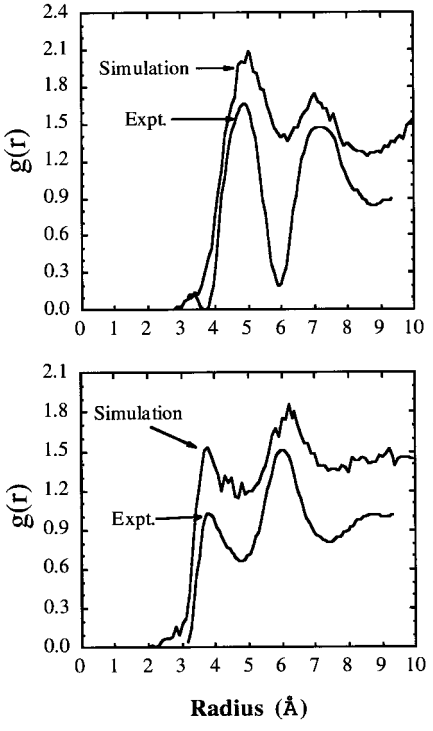

FIGURE 1 RDFs between carbon and chlorine atoms (*top*) and between chlorine and chlorine atoms (*bottom*) are shown for the 300-ps simulation of 256 carbon tetrachloride molecules and experiment.

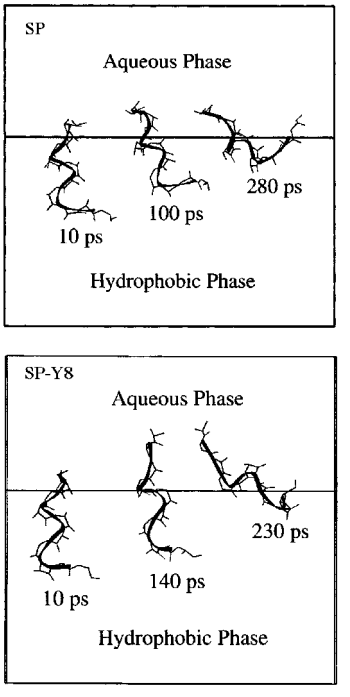

FIGURE 3 Equilibration of SP (*top*) and SP-Y8 (*bottom*) originally placed perpendicular to the interface. Interface boundary drawn at intersection of water and carbon tetrachloride densities. Only the backbone atoms are shown. The N-terminus starts out in the aqueous phase.

series for a normalized vector defined as the best fit helical cylindrical surface for the helical sections of SP (residues 4–8) and SP-Y8 (residues 3–7) are shown in Fig. 4 for all simulations. Fluctuations of the helical axis remain throughout the simulation after the peptides reach an essentially parallel orientation to the interface. These fluctuations should be expected considering the shortness of our defined helix and the sharpness of the interface. The faster equilibration for SP-Y8 was perhaps due to the more polar tyrosine as opposed to the hydrophobic phenylalanine. Both of the simulations with the perpendicular starting orientation revealed that a poor initial guess of the orientation and position of the peptide can be overcome in a reasonable amount of simulation time in the biphasic cell (see also Guba and Kessler, 1994). SP, originally placed in a parallel orientation, was continued for 190 ps past equilibration for a total restrained simulation time of 369 ps. SP-Y8, originally placed in a parallel orientation, was continued 226 ps past equilibration for a total restrained simulation time of 387 ps. The simulations were continued without restraints on the peptide for 400 ps for SP and 276 ps for SP-Y8. Coordinates from the trajectory were sampled every 200 fs for analysis.

Conformational/orientational properties

SP

In the following several sections the detailed analysis of the properties of the peptide/biphasic system is presented. The analysis is based on the simulations of the two peptides that were originally oriented parallel to the surface, because this orientation is closer to the equilibrated orientation. The conformation of SP is reflected in the ϕ-ψ values given in Table 2 for both the restrained and unrestrained MD simulations. SP is characterized by two type I β-turns (Wilmot

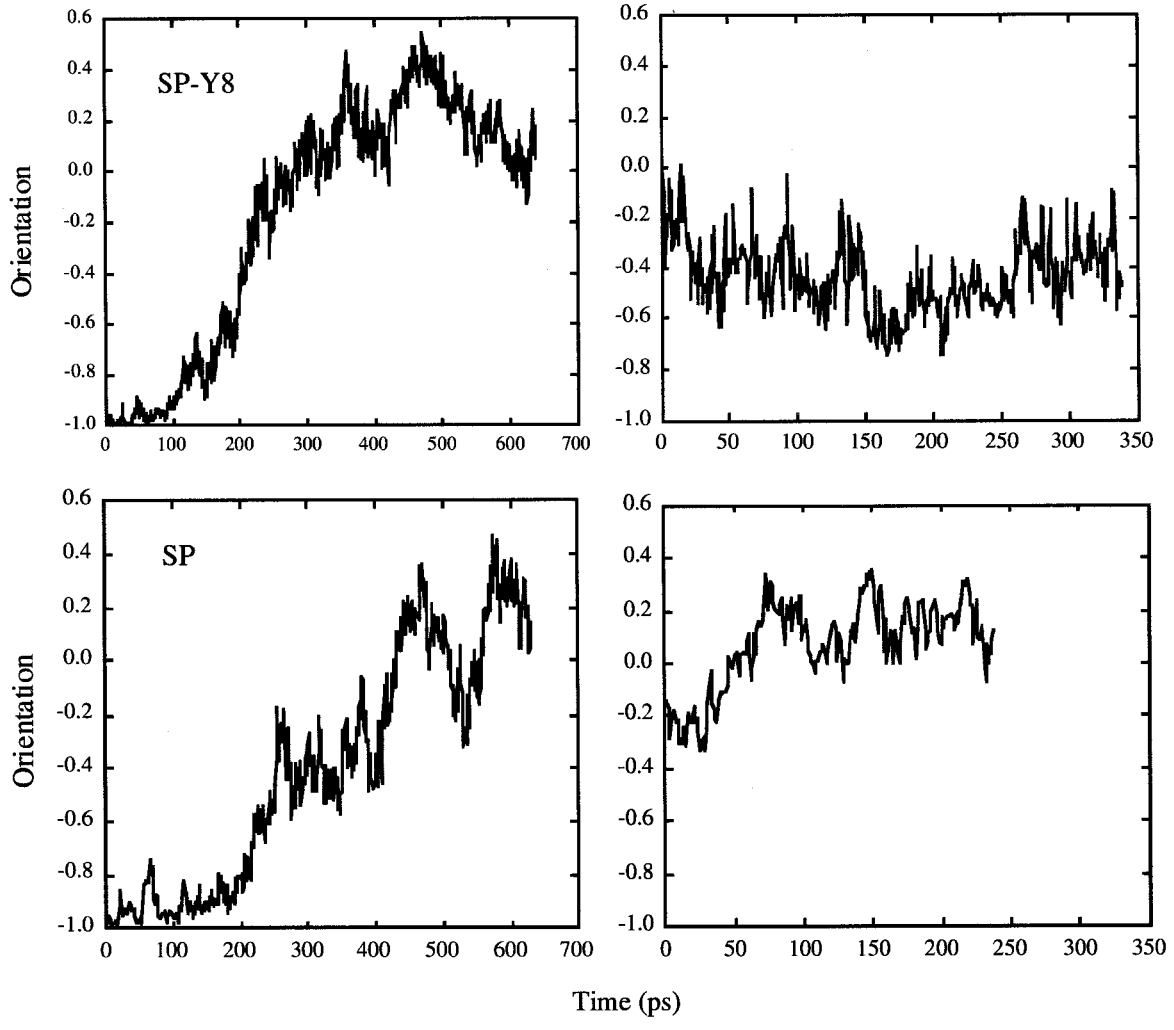

FIGURE 4 The orientational equilibration during restrained MD is shown by mapping a normalized vector defined as the best fit helical cylindrical surface fitting the backbone atoms from residues 4–8 in SP, and 3–7 in SP-Y8. A value of −1 indicates a perfectly perpendicular orientation with respect to the interface. SP-Y8 originally placed perpendicular (*top left*), SP-Y8 originally placed parallel (*top right*), SP originally placed perpendicular (*bottom left*), and SP originally placed parallel (*bottom right*). SP-Y8 originally placed parallel had an orientation that fluctuated and occasionally was perpendicular to the interface, but with this segment in the aqueous phase and not in an insertion mode.

TABLE 2 The average ϕ-ψ values for SP and SP-Y8 in biphasic cell during the RMD and MD simulations

	RMD		MD	
Residue	ϕ	ψ	ϕ	ψ
SP				
Arg-1	NA	159.8	NA	161.9
Pro-2	−79.7	−174.0	−68.0	160.9
Lys-3	−44.9	−49.3	−56.0	−49.6
Pro-4	−71.2	−3.1	−70.4	−16.8
Gln-5	−108.3	−4.0	−84.0	−32.5
Gln-6	−65.4	−32.8	−46.6	−46.4
Phe-7	−69.7	−9.7	−63.7	−52.7
Phe-8	−70.4	−7.5	−73.9	−21.7
Gly-9	−141.4	61.4	−78.1	−37.3
Leu-10	−35.3	121.3	−78.1	−54.1
Met-11	91.8	NA	82.3	NA
SP-Y8				
Arg-1	NA	153.0	NA	139.5
Pro-2	−64.5	177.5	−71.4	170.4
Lys-3	−94.9	−11.5	−71.5	−40.9
Pro-4	−72.0	−172.3	−63.4	146.4
Gln-5	38.7	−34.4	68.4	−74.8
Gln-6	−22.7	−65.8	−43.6	−33.6
Phe-7	−56.1	−72.8	−79.1	−54.6
Tyr-8	−60.2	71.0	−90.6	50.0
Gly-9	−179.6	39.4	−121.8	86.6
Leu-10	−75.5	171.0	−96.7	162.2
Met-11	74.6	NA	81.4	NA

TABLE 3 Hydrogen bonding patterns with average acceptor-donor hydrogen distance (in angstroms) and average acceptor-hydrogen donor angles (in degrees) for the RMD of SP and SP-Y8 (rms errors given in parentheses). See text for discussion of hydrogen bonding criteria

Acceptor	Donor	Distance (Å)	Angle (°)
SP			
Pro-2 O	Gln-5 HN	2.03 (0.16)	147.8 (8.4)
Lys-3 O	Gln-6 HN	2.16 (0.17)	126.7 (9.8)
Gln-5 O	Phe-8 HN	2.61 (0.31)	156.7 (6.4)
Gln-6 O	Gly-9 HN	2.65 (0.35)	161.1 (9.9)
Phe-7 O	Leu-10 HN	2.02 (0.15)	149.7 (10.5)
Gln-5 Oϵ	Arg-1 HH11*	2.75 (1.20)	140.2 (13.4)
SP-Y8			
Lys-3 O	Phe-7 HN	2.78 (0.28)	130.3 (8.7)
Pro-4 O	Gln-6 HN	1.79 (0.07)	146.2 (4.9)
Phe-7 O	Gly-9 HN	1.99 (0.11)	139.3 (7.2)
Leu-10 O	Met-11 HT2#	2.32 (0.53)	133.7 (19.4)

*HH11 and HH12 are protons bonded to one of the terminal amino groups on the Arg side chain.
#HT2 and HT1 are the protons bonded to the amidated C-terminus.

and Thornton, 1990). The first turn is between Pro-2 and Gln-5. The other turn is between Gln-5 and Phe-8. Another extended turn exists between Phe-7 to Leu-10/Met-11, but does not rigorously meet the criteria for any certain type of β-turn. This structure for SP is very similar to the one reported by Keire and Fletcher (1996). The carbonyl of the peptide backbone for residues 2–7 excluding residue 4 are all hydrogen-bonded to the $i + 3$ NH of the peptide backbone. The criteria for defining a hydrogen bond are an average acceptor-donor hydrogen distance of <2.8 Å and an average acceptor-hydrogen-donor angle $>120°$ (Ravishanker et al., 1994). The hydrogen bond data from the restrained MD (RMD) are given in Table 3. This conformation of SP is shown in the biphasic cell in a typical orientation in Fig. 5. This orientation shown in Fig. 5 remained throughout the simulation with the axis of the peptide backbone being essentially parallel to the interface. When the restraints are removed from SP, the peptide undergoes a conformational transition to α-helical spanning residues 3–10, which is completed within 100 ps. Some experimental evidence suggests that SP does not form such a helix in the presence of micelles (Keire and Fletcher, 1996), while another study reported that SP is helical from Pro-4 through Phe-8, but that the helical form fluctuates from 3_{10} to α-helical (Young et al., 1994). This conformational transition observed in the free MD may be understood when one considers the sharpness of the interface and the neglect of the headgroup interactions. The sharpness of the interface forces the backbone to lie closer to the hydrophobic phase in order to partition the nonpolar side chains into the hydrophobic phase than the peptide would in micelles/bilayers, where it has been shown that the interface is quite broad (Weiner and White, 1992). Since any representation of the micelle headgroup is neglected in this biphasic model,

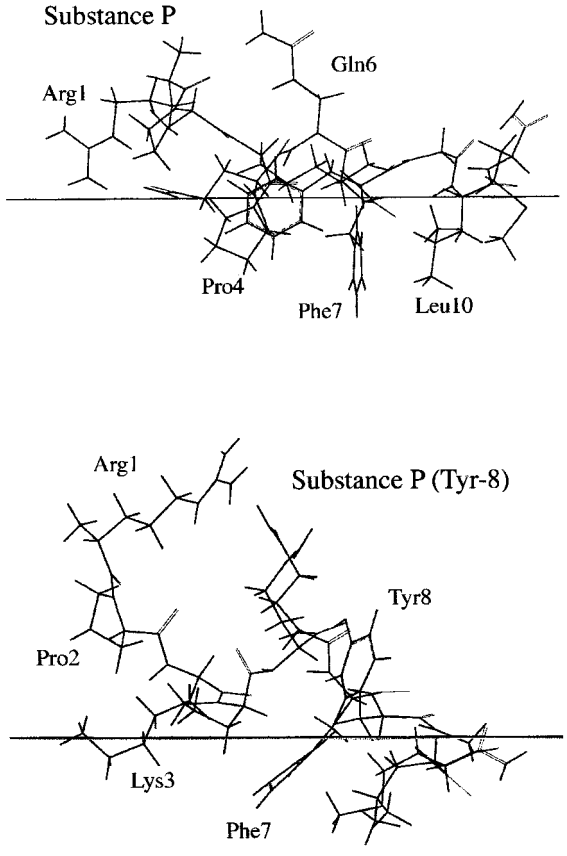

FIGURE 5 SP (*top*) and SP-Y8 (*bottom*) in a typical orientation and conformation in a biphasic cell. The phase above the horizontal line is the aqueous phase.

a helix lying on the surface does not perturb this interfacial area as it does in other membrane media (Dathe et al., 1996). Furthermore, a short peptide is likely to exhibit a large degree of flexibility even if somewhat immobilized on the surface of a micelle (Spyracopoulos et al., 1996).

SP-Y8

The ϕ-ψ values for SP-Y8 are given in Table 2. Residues Gln-6 and Phe-7 are shown to have values characteristic of an α-helical structure. SP-Y8 appears to have similar overall features of SP but the turn structures are more extended and do not fit any category of β-turn. The difference in the structure may be related to the interaction of the more polar tyrosine with water. SP has both phenylalanines immersed in the carbon tetrachloride, which forces this section to lie more parallel to the interface. Since this orientation does not allow as much interaction with water, the backbone will be more likely to form intramolecular hydrogen bonds, i.e., secondary structure. In comparison, Tyr-8 is well solvated by water, the orientation of the peptide is less parallel for this section, and the backbone has less of a need to form intramolecular hydrogen bonds. Since the interaction of the peptide with the headgroups in the membrane/water interface has been neglected in this model, there may be other reasons why these two similar peptides form slightly different secondary structures. Simulations in the biphasic system have the advantage of being able to correlate the differences in the secondary structures with the differences in the orientation of the peptide with respect to the water/membrane interface. When the restraints are removed from SP-Y8, secondary structure changes are observed (see Dynamics of the peptides section below). The conformation of SP-Y8 in a typical orientation from the RMD is shown in Fig. 5.

One major difference in the conformations of SP and SP-Y8 is in the relative orientations of the side chains of the two aromatic residues. In SP, both Phe-7 and Phe-8 are directed toward the hydrophobic phase (Fig. 5). There is probably stacking interaction between the two aromatic rings. In SP-Y8, however, because of the tendency of the hydroxyl group in the tyrosine ring to be in contact with water, the orientations of Phe-7 and Tyr-8 are almost in opposite directions (Fig. 5), pointing toward the hydrophobic and aqueous phases, respectively. The consequences of this difference in the orientation of these two peptides with respect to the interface, their binding affinity to membrane, and possible biological activities will be discussed in later sections.

Peptide/solvent properties

The analysis was carried out over the RMD section of the simulation. Because of the use of RMD trajectories, our results may be slightly biased in that the backbone will experience smaller fluctuations than in the case when the restraints are removed. This will in turn raise or lower the values in the radial distribution function (RDF). The overall picture should not be significantly different because of this, however, since those sections that are restrained are generally those involved in intramolecular hydrogen bonding, and secondary structures thus formed should not drastically change during a short simulation. To determine which residues interact with each respective phase, the aqueous or the hydrophobic, RDF, g(r), the associated hydration numbers, and the positions of the first peak (Table 4) were calculated between the carbonyl oxygen of the peptide backbone and oxygen atoms of TIP3P water. Another RDF was determined for key side chain atoms and the oxygen atoms of water. The need for the analysis of both of these two RDFs is that although a *side chain* may be hydrophobic, the peptide backbone is very polar. Thus the side chain and the backbone of the same residue may be partitioned in different phases. Wimley and White (1996) have determined that for interfacial peptides the backbone is more polar than any of the polar uncharged side chains. This suggests that hydrophobic residues on the surface may tend to insert just the side chain atoms of a residue into the hydrophobic region, while the backbone may prefer to be solvated or in a hydrogen-bonded environment.

SP

The carbonyl oxygen atoms of the polar peptide backbone are solvated or participating in intramolecular hydrogen bonds for SP (see Fig. 6 and Tables 3 and 4). In the RDF of most of the residues a prominent peak at ~3 Å appears, indicating a primary hydration shell around the carbonyl oxygen atom. Intramolecular hydrogen bonding reduces the sizes of the first peak in the RDF, and in the case of strong hydrogen bonding, the hydration peak in the RDF may completely disappear. As shown in Fig. 6, the RDF of Pro-2, Lys-3, Phe-7 showed no solvation peak with water, and these are also the residues that are involved in the strongest hydrogen bonds as judged by the short O–H distance (Table 3). The exception, i.e., there is neither a

TABLE 4 Hydration numbers for the SP peptide carbonyl oxygen atoms with oxygen atoms of water from RDFs shown in Fig. 6

Residue	First Peak Position (Å)	Integrated to (Å)	Hydration No.
Arg-1	3.3	4.5	1.44
Pro-2	—	**5.0**	**0.31**
Lys-3	—	**5.0**	**0.30**
Pro-4	—	5.0	0.00
Gln-5	2.7	3.4	0.61
Gln-6	2.8	3.4	0.91
Phe-7	—	**5.0**	**0.03**
Phe-8	2.8	3.4	0.92
Gly-9	2.8	3.4	1.05
Leu-10	2.8	3.4	0.84
Met-11	2.8	3.4	1.42

Residues in bold participate predominately in intramolecular hydrogen bonds.

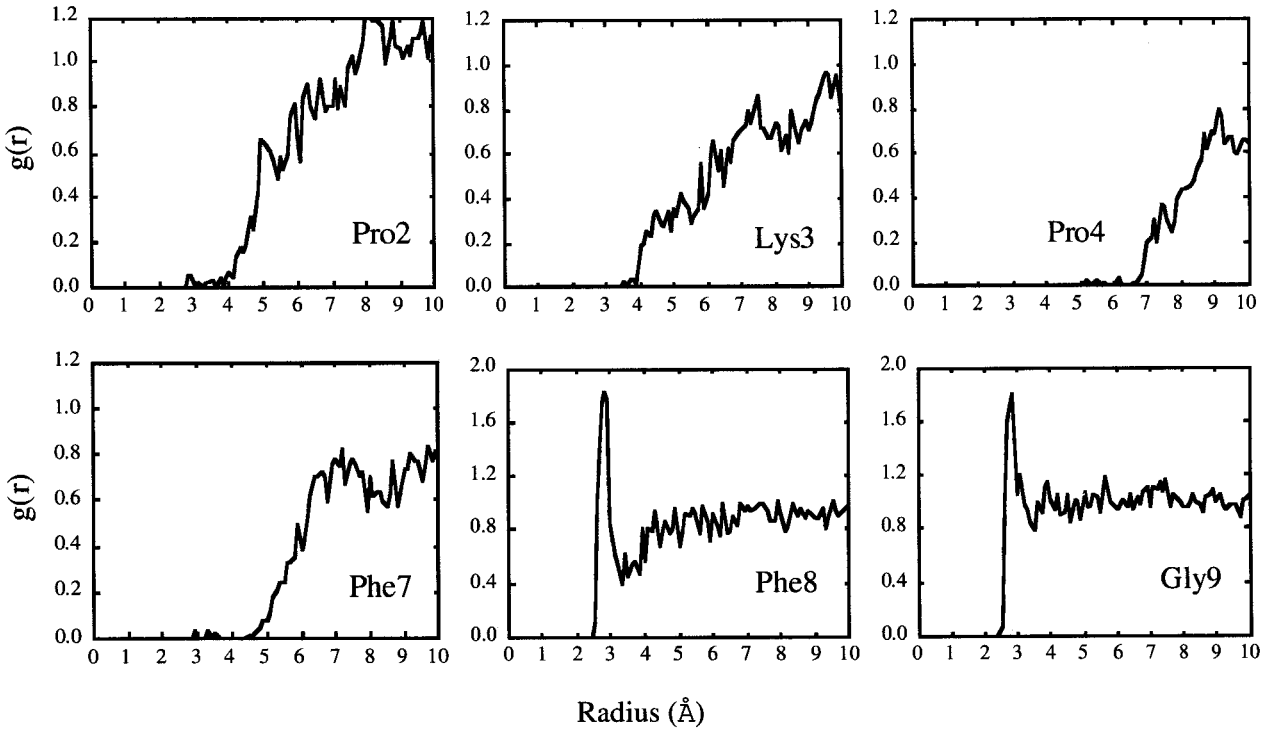

FIGURE 6 RDFs for selected SP backbone carbonyl atoms with the oxygen atoms of TIP3P water. Residues not shown have an RDF similar to that of Phe-8.

hydration peak in the RDF nor hydrogen bonding, is the carbonyl oxygen of Pro-4 which is found to be situated in a pocket formed by the peptide in close proximity to both aromatic side chains. In general, the simulations reinforce the conclusions from experiment (Wimley and White, 1996) that the peptide backbone is very polar and tends to remain either solvated or in a hydrogen-bonded environment, or both. Theoretical calculations (Ben-Tal et al., 1996) suggest that inserting the peptide bond into the hydrophobic phase even in the α-helical conformation is energetically unfavorable by 2.2 kcal per residue. The strong tendency for the peptide backbone to stay solvated by water is the primary reason for peptides to interact with the membrane via the hydrophobic side chains. Thus, it is unlikely to see small peptides to be inserted into the membrane even when the peptide possesses primary amphiphilicity, i.e., possessing distinct charged and hydrophobic segments, as it was proposed by Schwyzer (1992).

The side chains that make a hydrophobic contribution to partitioning are Pro-4, Phe-7, Phe-8, Leu-10, and Met-11. The RDFs for side chain carbon atoms with the oxygen atoms of water are shown in Fig. 7. Unlike the case for the peptide backbone, where if the carbonyl oxygen did not show a solvent peak it was still in a polar environment due to intramolecular hydrogen bonding, the side chain carbon atoms that do not display a water solvent peak in the RDF

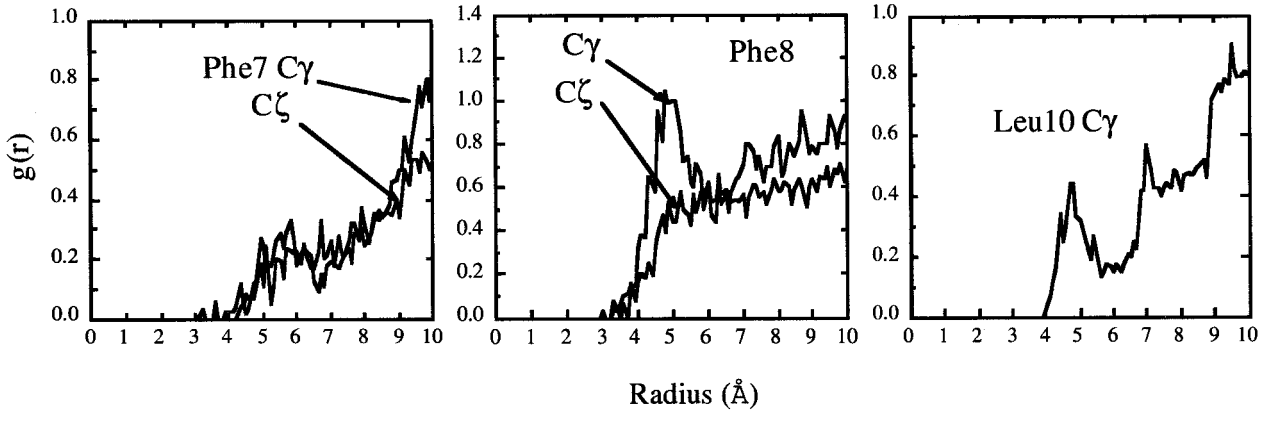

FIGURE 7 RDFs of selected hydrophobic side chain atoms with the oxygen atoms of TIP3P water.

are truly in a hydrophobic region. Thus, the side chains of Phe-7, Phe-8, Leu-10, and Met-11 showed no solvation by water, as shown by the RDFs in Fig. 7, indicating that these side chains reside in the hydrophobic phase. Furthermore, the Lys-3 side chain is shown to make a small hydrophobic contribution shown in Fig. 8. This is the so-called "snorkel effect" (Segrest et al., 1990) where the methylenes are in the hydrophobic region with the charged amino group pulled toward the water. This effect is quite minor in the biphasic cell but does have a bigger effect when the simulation is performed with an explicit micelle (see following paper).

SP-Y8

The same analysis was applied to SP-Y8. Again the results showed that all the carbonyls of the peptide backbone were either solvated (Fig. 9 and Table 5) or in a hydrogen-bonded environment (Table 3), providing further support for the importance of the peptide backbone in determining the partitioning of the peptide. Phe-7 has a high hydration number and is also involved in intramolecular hydrogen bonding. This high hydration number is due to the broader first hydration peak and the peak has to be integrated further out (to 4.4 Å) to determine this hydration number. SP-Y8 has more backbone atoms solvated, suggesting that hydrophobic interactions make a smaller contribution to partitioning than does SP, consistent with the values of the ΔG_{part} for SP and SP-Y8 measured in this laboratory (Wong and Gao, 1998). The intramolecular hydrogen bonding is reduced from SP as shown in Table 3, a result of the higher solvation of the backbone by water. The side chains of SP-Y8 are more solvated by water than SP, most notably the comparison between Phe-8 of SP and Tyr-8 of SP-Y8 (Fig. 10). The different hydrophobicities in these two peptides can be further visualized by examining Fig. 11, which shows the close contact of each side chain with molecules of both solvents. The replacement of Phe-8 by the less hydrophobic Tyr not only results in a higher solvation (by water) of the Tyr side chain; there is also an overall increase in the solvation of most of the side chains in SP-Y8 by water, Pro-4, and Gln-5, in particular.

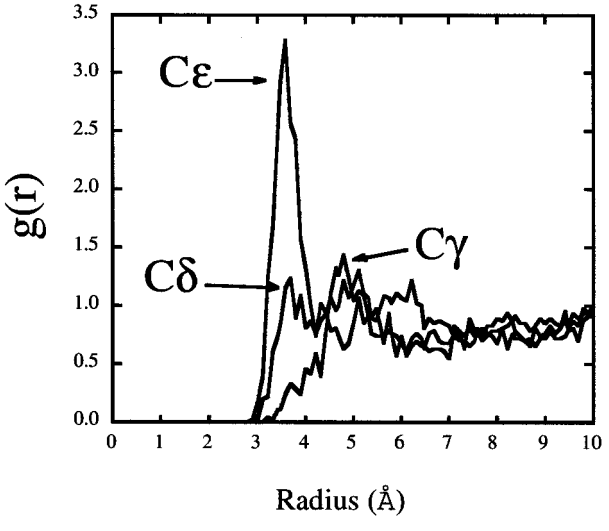

FIGURE 8 RDFs for side chain atoms of Lys-3 with the oxygen atoms of TIP3P water demonstrating the snorkel effect. N_ζ is not shown due to the magnitude of first oxygen water peak.

Dynamics of the peptide

SP

The trajectory of SP was analyzed over the unrestrained MD from 128–400 ps. During the simulation time preceding this, SP changed its conformation slightly from two consecutive type I β-turns and an extended type turn to a conformation close to an α-helix. The concept of the amphipathic helix (Kaiser and Kezdy, 1983) is an important one in protein-membrane interactions. The interaction of the hydrophobic side of the helix with the hydrophobic core is a primary force in determining the orientation of the helix with respect to the membrane surface. SP actually had more side chain atoms inserted into the hydrophobic region before the conformational transition. By making this transition, Leu-10 becomes immersed in the aqueous region but this section of the peptide gains intramolecular hydrogen bonds to compensate for the change. Interestingly, during this time period of unrestrained simulation SP undergoes two conformational transitions but quickly returns to the primary conformation. The first conformational transition occurs at 328 ps of the unrestrained dynamics (200 ps after the transition to α-helix) with dihedral angles of ψ Gln-6 and ϕ Phe-7. An examination of probable causes of the cooperative nature of these two dihedral angle transitions reveals that if only Gln-6 ψ changes it will force Phe-8 into the aqueous phase; if only Phe-7 ϕ changes it will force Phe-7 into the aqueous phase. These isolated changes in dihedral angles would be undesirable because the phenylalanine residues have a clear preference for the hydrophobic phase. The second conformational transition occurs at 353 ps of the unrestrained dynamics (225 ps after the transition to α-helix) involving the ψ of Phe-8 and the ϕ of Gly-9. This second conformational transition also lasts for only 10–15 ps and the peptide returns to the α-helical structure. The cooperative nature of these transitions appears to be due to conserving a turn structure from Phe-7 to Met-11, which is necessary to keep the corresponding backbone hydrogen-bonded, thus allowing these residues to be better immersed into the hydrophobic phase.

SP-Y8

SP-Y8 was also simulated without restraints for 276 ps. SP-Y8 undergoes a concerted dihedral angle transition involving Gln-6 ψ and Phe-7 ϕ, which results in forming a turn from Lys-3 to Gln-5. Side chain positions with respect to the respective phases are unaffected. The most interesting transition comes from Tyr-8 ψ/Gly-9 ψ. The Tyr-8 ψ tran-

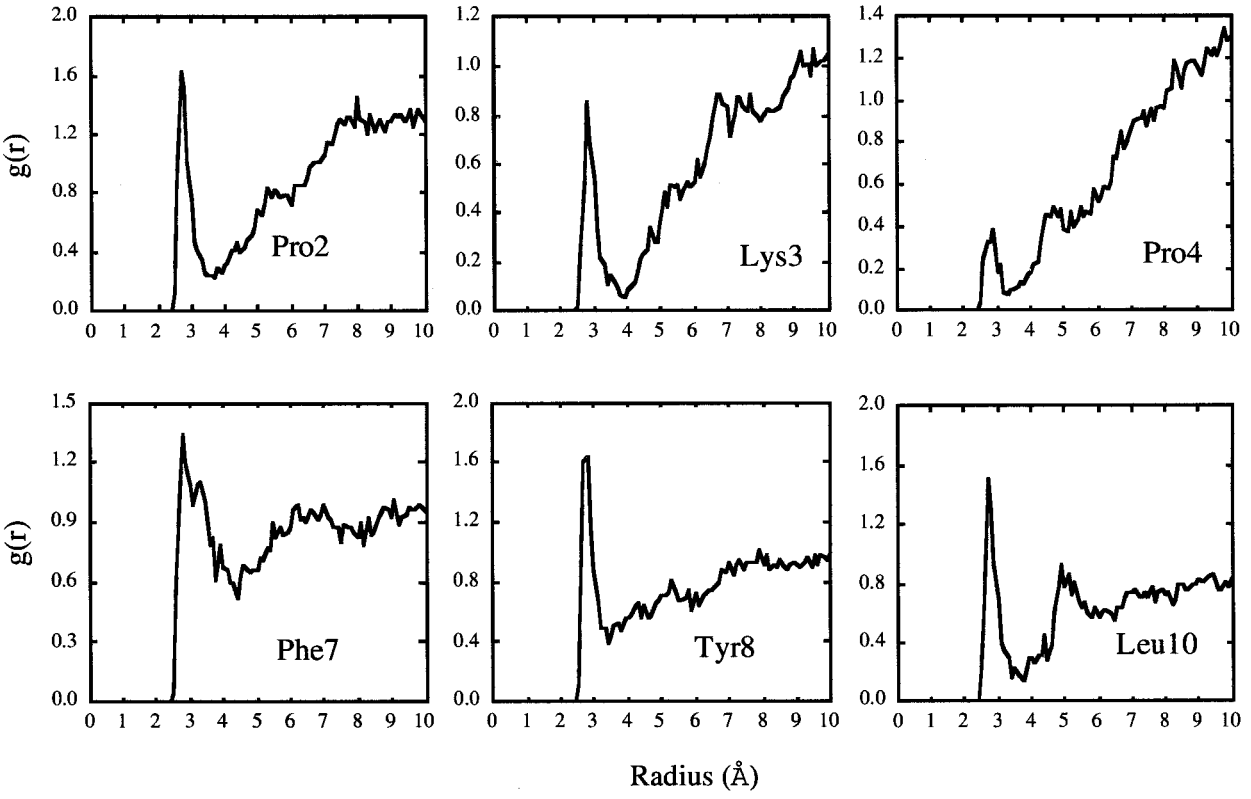

FIGURE 9 RDFs for selected backbone carbonyl atoms of SP-Y8 with the oxygen atoms of TIP3P water. Residues not shown have an RDF similar to that of Tyr-8.

sition results in Tyr-8 being immersed into the hydrophobic phase with the hydroxyl group of Tyr-8 forming a hydrogen bond with Gln-5 NH of the peptide backbone. The concerted Gly-9 ψ transition results in preserving the Leu-10/Met-11 side chain positions in the hydrophobic phase and in the loss of the extended turn from Phe-7 to Met-11. The overall result of the Tyr-8 ψ/Gly-9 ψ transition is that both aromatic residues are now in the hydrophobic phase, with the hydrophobic side chains Leu-10 and Met-11 remaining in the hydrophobic phase.

TABLE 5 Hydration numbers for the SP-Y8 peptide carbonyl oxygen atoms with oxygen atoms of water from RDFs shown in Fig. 9

Residue	First Peak Position (Å)	Integrated to (Å)	Hydration No.
Arg-1	3.0	4.5	2.15
Pro-2	2.7	3.4	0.72
Lys-3	2.8	3.4	0.36
Pro-4	**2.9**	**3.4**	**0.20**
Gln-5	2.7	3.4	0.64
Gln-6	2.8	3.4	1.07
Phe-7	**2.8**	**4.4**	**1.64**
Tyr-8	2.8	3.4	0.81
Gly-9	2.8	3.4	1.37
Leu-10	**2.7**	**3.4**	**0.64**
Met-11	2.7	3.4	1.37

Residues in bold are predominately in intramolecular hydrogen bonds.

The conformations of SP that deviated from α-helical last only ~10–15 ps, while those of SP-Y8 lasted much longer. Dihedral angle transitions may occur in the biphasic cell on a shorter time scale than in micellar solution because the carbon tetrachloride phase does not immobilize the peptide on the time scale of this simulation. The synchronous transitions mentioned above are due to the nature of the biphasic environment in that the hydrophobic side chains will want to remain in the hydrophobic phase. This process is untenable if only one dihedral angle transition occurs. Observation of different possible conformations is important when considering that results from the biphasic cell can be used to build initial configurations for simulations in micellar media. Since conformational transitions may not occur in lipid bilayers or micelles on a simulation time scale of around a nanosecond, these alternate conformations could be used to run several simulations to get a more complete picture of the specific peptide-micelle complex (Damodaran et al., 1995).

Comparison to experiments done in membrane media

For SP in micelles there are many experimental studies with which our simulation results can be compared. Fluorescence of the Phe residues (283 nm) increases twofold in micelles, indicating changes to a more hydrophobic and/or a less mobile environment upon interaction with the micelles

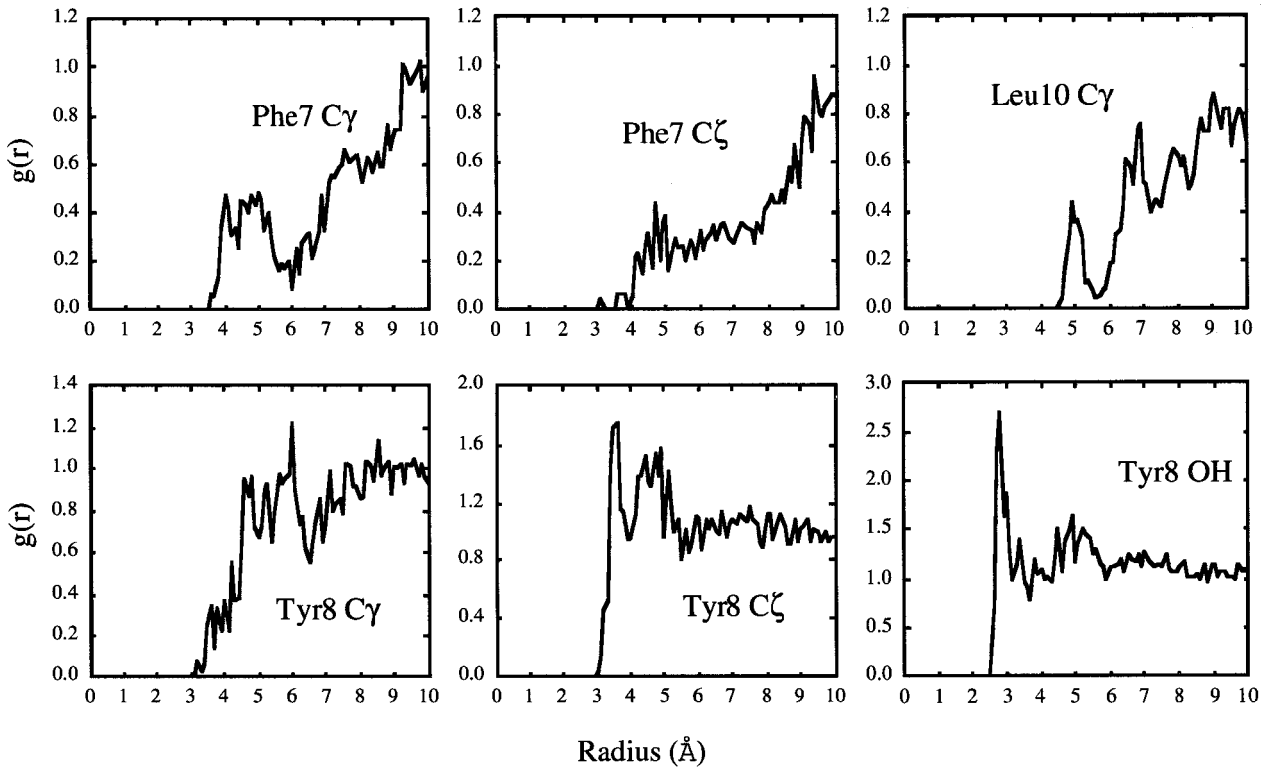

FIGURE 10 RDFs of selected side chain atoms of SP-Y8 with the oxygen atoms of TIP3P water.

(Woolley and Deber, 1987). The SP simulations clearly showed this feature. In fact, the interaction of the Phe residues seems to be the central hydrophobic interaction, since during the simulation Leu-10 undergoes a transition to the aqueous phase while neither Phe-7 nor Phe-8 does. NMR NOESY spectra showed crosspeaks between the methylenes of SDS micelle and the hydrogen atoms of the phenylalanine rings, which give further evidence for the insertion of these side chains (Hicks et al., 1992). This same study could not identify any NOE correlations between Leu-10 and the methylene hydrogen atoms of SDS. This may be due to the dynamic nature of the peptide and or lipids, yet NOESY crosspeaks exist between the Leu-10 side chain and the aromatic hydrogen atoms of Phe and Tyr (Keire and Fletcher, 1996; Gao and Wong, submitted for publication), which is only possible if these residues are on the same face interacting with the micelle. Our RMD simulations also indicate evidence of Leu-10 insertion into the micelle due to the fact that this residue is residing in the carbon tetrachloride phase during the majority of the simulation. Duplaa et al. (1992), based on ^{13}C-NMR and surface potential measurements, suggested two models for SP interacting with the membrane, in one of which the two Phe residues were inserted into the membrane and the other model had the last three residues inserted. The latter model seems unlikely because of the need for the desolvation of the last three peptide bonds. The simulation results suggest that our model may be similar to the one suggested by Duplaa et al. (1992) in that both Phe residues are inserted with the helical structure as in the MD and another that has the Phe residues and Leu-10 side chains inserted with the conformation as used in the RMD. Clearly, the present simulation result is not in agreement with the model proposed by Schwyzer (1992) in which SP is inserted perpendicularly to the water/membrane interface with nine residues at the N-terminus residing in the hydrophobic region, based on attenuated total reflectance infrared spectroscopy (ATR-IR) and capacitance minimization measurements. There is some (albeit weak) evidence from NMR that SP undergoes rapid conformational exchange (Young et al., 1994) between an α-helix and a 3_{10} helix in SDS micelles, but whether this is correlated with Leu-10 side chain insertion is difficult to determine. Lastly, the position of the charged and polar residues show a strong preference for the aqueous phase, as would be expected.

The orientation of SP in the biphasic cell equilibrates to a parallel orientation, which is consistent with most experimental data and theoretical studies, in simulations starting from very different orientations. For SP to insert into the lipid core in a perpendicular fashion would require that some of the C-terminal peptide bonds lose hydrogen bonds to water and not regain them unless the peptide is able to curl around in some fashion to hydrogen-bond to the side chains (see Ben-Tal et al., 1996). Since there is no evidence that this structure occurs, it is consistent with other theoretical calculations (Ben-Tal et al., 1996) that SP and SP-Y8 must lie on the surface of a hydrophobic/hydrophilic interface.

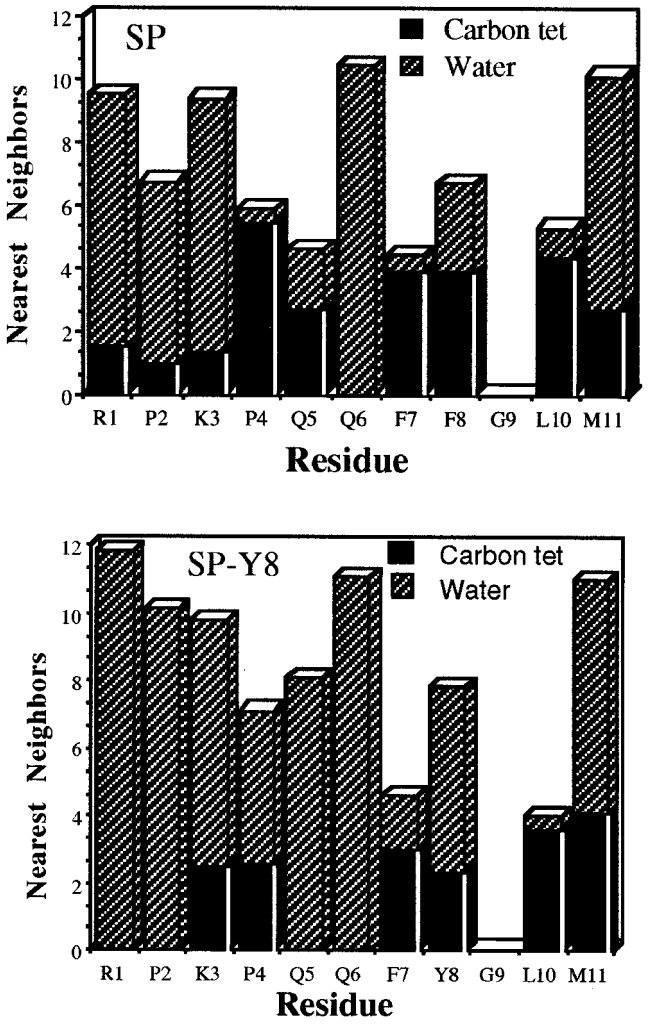

FIGURE 11 Contributions from the biphasic cell to the solvation of the side chains of SP (*top*) and SP-Y8 (*bottom*). The average solvation number was calculated by counting the number of nearest neighbors within a distance of 5 Å around each side chain, averaged over the last 60 ps of the trajectory, and normalized with respect to the total number of heavy atoms (excluding hydrogen) in each side chain.

Changing the eighth residue on SP from the hydrophobic phenylalanine to a more polar tyrosine has many consequences for the MD in the biphasic cell. Since the side chain aromatic insertion into the hydrophobic phase is the major hydrophobic interaction, substitution of a more polar residue (such as Tyr) forces the peptide to orient at an angle to the interface for residues 1–8. The studies of Wimley et al. (1996) and Thorgeirsson et al. (1996) showed that the free energy of partitioning into lipid bilayer, ΔG_{part}, should be more negative for Phe than for Tyr by ~1 kcal/mol. This difference was qualitatively corroborated by a partition study from this laboratory for SP and SP-Y8 in dodecylphosphocholine (DPC) micelles (Wong and Gao, 1998). The partition coefficient for SP in DPC micelles was found to be about twice that of SP-Y8 at 298 K, corresponding to a difference in the ΔG_{part} of ~0.35 kcal/mol. The difference ΔG_{part} mainly arises from the different degree of hydrophobic interaction (with the membrane) of Phe and Tyr.

CONCLUSIONS

The use of the biphasic cell in the study of SP peptide orientation and conformation has been shown to reproduce experimental observations of SP and SP-Y8 in membrane media. The biphasic cell is seen to work best with peptides with a significant interaction with the methylenes of lipids since it is this interaction that most closely resembles lipid bilayers or micelles. Another important aspect is the fact that simulations can be done relatively quickly compared to simulations in explicit membrane media. The simulation time needed for equilibration even when the peptide is placed in an unfavorable initial orientation/position is small (~250 ps), and reorientation and repositioning of the peptide are possible in this system. This is an important ingredient because the orientation and position of a peptide with respect to the membrane are frequently difficult to determine experimentally. However, the results of this work also point out the danger of doing MD simulation over a short time period (<300 ps). When the simulation time is too short for the system to equilibrate, the apparent results (conformation, orientation, and position) will be strongly biased by the choice of the initial configuration. Such is the case, we believe, in a MD simulation of SP in a lipid bilayer for 260 ps (Kothekar, 1996).

The biphasic cell also correctly places the most hydrophobic residues in the hydrophobic phase. Furthermore, the results seem consistent with theoretical (Ben-Tal et al., 1996) and experimental results (Wimley and White, 1996) in that the peptide backbone is very polar and therefore is expected to either be solvated or participate in intramolecular hydrogen bonding and be oriented at the surface. The results from these simulations can also be used to construct favorable initial configurations for peptides in explicit membrane models where the amount of simulation time needed for equilibration is expected to be longer (see following paper) and is thus of a major concern in terms of computational time.

Comparing the two SP peptides that differ by only a hydroxyl group on the eighth residue, different sets of properties emerge. SP appears to have a larger hydrophobic interaction, the strongest of which comes from the side chains of the two aromatic residues; its orientation is nearly parallel to the interface while SP-Y8 has a smaller interaction with the hydrophobic phase, and its orientation is more tilted away from the parallel orientation, though not in an insertion mode. The two aromatic side chains (Phe-7 and Tyr-8) are pointed in opposite directions—Tyr-8 is solvated by water while Phe-7 is mostly in the hydrophobic phase—and show no stacking interaction. The different properties exhibited by the two peptides may explain their different biological properties; SP-Y8 being a weaker agonist of the NK1 receptor (Fisher et al., 1976). The two aromatic resi-

dues of SP have been shown to be important for receptor recognition and activation (Huang et al., 1994). Both aromatic side chains have been shown to be especially attracted to the bilayer interfacial region (Wimley and White, 1996), but with different hydrophobicities. Thus it may be argued that the difference in the biological properties of these two peptides may be in how they interact with the *lipids* before interacting with the receptor, according to the membrane-mediated mechanism (Schwyzer, 1992). One needs to be cautious in the interpretation of such results because our simulations show that changing a residue's hydrophobicity can change not only the secondary structure, which may be related to the "bioactive conformation," but the orientation, position, and binding affinity of the peptide to the membrane.

Not only is the result of the present study useful as a mimic for amphiphilic peptides in membrane, it is also relevant to the study of amphiphilic molecules at liquid-liquid interfaces. Few if any experimental evidence exists for peptides at the water/carbon tetrachloride interface. Nonlinear optical techniques (sum-frequency generation and second harmonic generation) are potentially powerful techniques for such purposes (Paul and Corn, 1997; Conboy et al., 1996). The study of peptides at interfaces with these methods may be a logical step in determining an interfacial hydrophobic index that would be free from the electrostatic interaction with the charged headgroups.

This work was supported by Grant MCB950034P from the Pittsburgh Supercomputing Center; sponsored by the National Science Foundation (NSF), and by a grant from the Research Council of the University of Missouri, Columbia. The support by Dr. Hossein Tahani and the University of Missouri Campus Computing is also gratefully acknowledged.

REFERENCES

Area, E. P., P. G. Pascutti, S. Schreier, K. Mundim, and P. M. Bisch. 1995. Molecular dynamics simulations of signal sequences at a membrane/water interface. *J. Phys. Chem.* 99:14885–14892.

Ben-Tal, N., A. Ben-Shaul, A. Nicholls, and B. Honig. 1996. Free-energy determinants of α-helix insertion into lipid bilayers. *Biophys. J.* 70: 1803–1812.

Ben-Tal, N., D. Sitkoff, I. Topol, A. Yang, S. Burt, and B. Honig. 1997. Free energy of amide hydrogen bond formation in vacuum, in water, and in liquid alkane solution. *J. Phys. Chem. B.* 101:450–457.

Bernèche, S., M. Nina, and B. Roux. 1998. Molecular dynamics simulation of melittin in a dimyristoylphosphatidylcholine bilayer membrane. *Biophys. J.* 75:1603–1618.

Biggin, P. C., J. Breed, H. S. Son, and M. S. P. Sansom. 1997. Simulation studies of alamethicin-bilayer interactions. *Biophys. J.* 72:627–636.

Bremi, T., R. Bruschweiler, and R. R. Ernst. 1997. A protocol for the interpretation of side-chain dynamics based on NMR relaxation: application to phenylalanines in antamanide. *J. Am. Chem. Soc.* 119: 4272–4284.

Brooks, B. R., R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus. 1983. CHARMM: a program for macromolecular energy, minimization, and dynamics calculations. *J. Comp. Chem.* 4:187–217.

Brooks, C. L. III, M. Karplus, and B. M. Pettitt. 1988. Proteins: a theoretical perspective of dynamics, structure, and thermodynamics. *Adv. Chem. Phys.* Vol. 71.

Brown, J., and W. Huestis. 1993. Structure and orientation of a bilayer-bound model tripeptide. A ^{1}H-NMR study. *J. Phys. Chem.* 97: 2967–2973.

Chang, T.-M., and L. X. Dang. 1996. Transfer of CH_4-CCl_4 liquid-liquid interface with polarizable potential models. *Chem. Phys. Lett.* 263: 39–45.

Conboy, J. C., M. C. Messmer, and G. L. Richmond. 1996. Investigation of surfactant conformation and order at the liquid-liquid interface by total internal reflection sum-frequency vibrational spectroscopy. *J. Phys. Chem.* 100:7617–7622.

Damodaran, K. V., and K. M. Merz. 1995. Interaction of the fusion inhibiting peptide carbobenzoxy-D-Phe-L-Phe-Gly with N-methyldioleoylphosphatidylethanolamine lipid bilayers. *J. Am. Chem. Soc.* 117: 6561–6571.

Damodaran, K. V., K. M. Merz, and B. P. Gaber. 1995. Interaction of small peptides with lipid bilayers. *Biophys. J.* 69:1299–1308.

Dathe, M., M. Schumann, T. Wieprecht, A. Winkler, M. Beyermann, E. Krause, K. Matsuzaki, O. Murase, and M. Bienert. 1996. Peptide helicity and membrane surface charge modulate the balance of electrostatic and hydrophobic interactions with lipid bilayers and biological membranes. *Biochemistry*. 35:12612–12622.

Duplaa, H., O. Convert, A.-M. Sautereau, J.-F. Tocanne, and G. Chassaing. 1992. Binding of substance P to monolayers and vesicles made of phosphatidylcholine and/or phophatidylserine. *Biochim. Biophys. Acta.* 1107:12–22.

Erne, D., K. Rolka, and R. Schwyzer. 1986. Membrane structure of substance P III. Secondary structure of substance P in 2,2,2-trifluoroethanol, methanol, and on flat lipid membrane studied by infrared spectroscopy. *Helv. Chim. Acta*. 69:1807–1816.

Essex, J. W., C. A. Reynolds, and W. G. Richards. 1992. Theoretical determination of partition coefficients. *J. Am. Chem. Soc.* 114: 3634–3639.

Fisher, G., K. Folkers, B. Pernow, and C. Bowers. 1976. Synthesis and some biological activities of the tyrosine-8 analog of substance P. *J. Med. Chem.* 19:325–328.

Frey, S., and L. K. Tamm. 1991. Orientation of melittin in phospholipid bilayers: a polarized attenuated total reflection infrared study. *Biophys. J.* 60:922–930.

Gao, X.-F., and T. C. Wong. 1998. Studies of the binding and structure of adrenocorticotropin peptides in membrane mimics by NMR spectroscopy and pulsed-field gradient diffusion. *Biophys. J.* 74:1871–1888.

Guba, W., R. Haessner, G. Breipohl, S. Henke, J. Knolle, V. Santagada, and H. Kessler. 1994. Combined approach of NMR and molecular dynamics within a biphasic membrane mimetic. Conformation and orientation of the bradykinin antagonist Hoe. 140. *J. Am. Chem. Soc.* 116:7532–7540.

Guba, W., and H. Kessler. 1994. A novel computational mimetic of biological membranes in molecular dynamics simulations. *J. Phys. Chem.* 98:23–27.

He, K., S. J. Ludtke, W. T. Heller, and H. W. Huang. 1996. Mechanism of alamethicin insertion into lipid bilayers. *Biophys. J.* 71:2669–2679.

Hicks, R. P., D. J. Beard, and J. K. Young. 1992. The interactions of neuropeptides with membrane model systems: a case study. *Biopolymers*. 32:85–96.

Huang, P., and G. H. Loew. 1995. Interaction of an amphiphilic peptide with a phospho-lipid bilayer surface by molecular dynamics simulation study. *J. Biomol. Struct. Dyn.* 12:937–956.

Huang, R. R. C., H. Yu, C. Strader, and T. M. Fong. 1994. Interaction of substance P with the second and seventh transmembrane domains of the Neurokinin-1 receptor. *Biochemistry*. 33:3007–3013.

Jacobs, R., and S. H. White. 1989. The nature of the hydrophobic binding of small peptides at the bilayer interface: implications for the insertion of transbilayer helices. *Biochemistry*. 28:3421–3437.

Jorgenson, W. L., R. W. Impey, J. Chandrasekhar, J. D. Madura, and M. L. Klein. 1983. Comparison of simple potential functions for simulating liquid water. *J. Chem. Phys.* 79:926–935.

Kaiser, E. T., and F. J. Kezdy. 1983. Secondary structure of proteins and peptides in amphiphilic environments. *Proc. Natl. Acad. Sci. USA*. 80:1137–1140.

Keire, D., and T. Fletcher. 1996. The conformation of substance P in lipid environments. *Biophys. J.* 70:1716–1727.

Kothekar, V. 1996. 260-ps molecular dynamics simulation of substance P with hydrated dimyristoyl phosphatidyl choline bilayer. *J. Biomol. Struct. Dyn.* 13:601–613.

Ludtke, S., W. T. Heller, T. A. Harroun, L. Yang, and H. W. Huang. 1996. Membrane pores induced by magainin. *Biochemistry*. 35:13723–13728.

MacKerell, Jr., A. D., D. Bashford, M. Bellott, R. L. Dunbrack, Jr., J. Evanseck, M. J. Field, S. Fischer, J. Gao, H. Guo, S. Ha, D. Joseph-McCarthy, L. Kuchnir, K. Kuczera, F. T. K. Lau, C. Mattos, S. Michnick, T. Ngo, D. T. Nguyen, B. Prodhom, W. E. Reiher III, B. Roux, M. Schlenkrich, J. C. Smith, R. Stote, J. Straub, M. Watanabe, J. Wiorkiewicz-Kuczera, D. Yin, and M. Karplus. 1998. All-atom empirical potential for molecular modeling and dynamics studies of protein. *J. Phys. Chem. B*. 102:3586–3616.

Mercurio, E., M. Pellegrini, and D. F. Mierke. 1997. Structure, dynamics, and topological orientation of the polyether, ionophore antibiotic monensin, in a micellar environment. *Biopolymers*. 42:759–769.

Merz, Jr., K. M., and B. Roux. 1996. Biological membranes: a molecular perspective from computation and experiment. Birkhauser, Boston.

Moroder, L., R. Romano, W. Guba, D. F. Mierke, H. Kessler, C. Delporte, J. Winand, and J. Christophe. 1993. New evidence for a membrane-bound pathway in hormone receptor binding. *Biochemistry*. 32: 13551–13559.

Narten, A. H. 1976. Liquid carbon tetrachloride: atom pair correlation functions from neutron and x-ray diffraction. *J. Chem Phys.* 65:573–579.

Nicoll, R. A., C. Schenker, and S. E. Leeman. 1980. Substance P as a transmitter candidate. *Ann. Rev. Neurosci.* 3:227–268.

Oda, K., H. Miyagawa, and K. Kitamura. 1996. How does the electrostatic force cut-off generate non-uniform temperature distributions in proteins? *Mol. Sim.* 16:167–177.

Opella, S. J. 1997. NMR and membrane proteins. *Nat. Struct. Biol., NMR Suppl*. 845–848.

Palmer III, A. G., J. Williams, and A. McDermott. 1996. Nuclear magnetic resonance studies of biopolymer dynamics. *J. Phys. Chem.* 100: 13293–13310.

Paul, H. J., and R. M. Corn. 1997. Second-harmonic generation measurements of electrostatic biopolymer-surfactant coadsorption at the water/1,2-dichloroethane interface. *J. Phys. Chem. B*. 101:4494–4497.

Pellegrini, M., and D. F. Mierke. 1997. Threonine^6-bradykinin: molecular dynamics simulation in a biphasic membrane mimetic. *J. Med. Chem.* 40:99–104.

Peng, J., C. Schiffer, P. Xu, W. F. van Gunsteren, and R. R. Ernst. 1996. Investigations of peptide hydration using NMR and molecular dynamics simulation: a study of effects of water on the conformation and dynamics of antamanide. *J. Biomol. NMR*. 8:453–456.

Philippopoulos, M., A. Mandel, A. G. Palmer III, and C. Lim. 1997. Accuracy and precision of NMR relaxation experiments and MD simulations for characterizing protein dynamics. *Proteins: Struct., Funct., Genet.* 28:481–493.

Pohorille, A., P. Cieplak, and M. A. Wilson. 1996. Interactions of anesthetics with the membrane-water interface. *Chem. Phys.* 204:337–345.

Ravishanker, G., S. Vijakumar, and D. L. Beveridge. 1994. STRIPS: an algorithm for generating two-dimensional hydrogen-bond topology diagrams for proteins. *In* Modeling the Hydrogen Bond. American Chemical Society, Washington, DC. 209–219.

Regoli, D., A. Bouden, and J. L. Fauchere. 1994. Receptors and antagonists for substance P and related peptides. *Pharmacol. Rev.* 46:551–599.

Regoli, D., S. Dion, N-E. Phaleb, N. Rouissi, C. Tousignant, D. Jukic, P. d'Orleans-Juste, and G. Drapeau. 1989. Selective agonists for receptors of substance P and related neurokinins. *Biopolymers*. 28:81–90.

Roseman, M. A. 1988. Hydrophobicity of the peptide C=O–H-N hydrogen bonded group. *J. Mol. Biol.* 201:621–625.

Ryckaert, J.-P., G. Cicotti, and H. J. C. Berendsen. 1977. Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. *J. Comput. Phys.* 23: 327–341.

Sargent, D. F., and R. Schwyzer. 1986. Membrane lipid phase as catalyst for peptide-receptor interactions. *Proc. Natl. Acad. Sci. USA*. 83: 5774–5778.

Schwyzer, R. 1992. Conformations and orientations of amphiphilic peptides induced by artificial lipid membranes: correlations with biological activity. *Chemtracts–Biochem. and Mol. Biol.* 3:347–379.

Schwyzer, R. 1995. 100 Year lock-and-key concept: are peptide keys shaped and guided to their receptors by the target cell membrane? *Biopolymers*. 37:5–16.

Seelig, A., and P. M. Macdonald. 1989. Binding of a neuropeptide, substance P, to neutral and negatively charged lipids. *Biochemistry*. 28: 2490–2496.

Segrest, J. P., H. De Loof, J. G. Dohlmann, C. G. Brouillette, and G. M. Anantharamaiah. 1990. Amphipathic helix motif: classes and properties. *Proteins: Struct., Funct., Genet.* 8:103–117.

Shen, L., D. Bassolino, and T. Stouch. 1997. Transmembrane helix structure, dynamics, and interactions: multi-nanosecond molecular dynamics simulations. *Biophys. J.* 73:3–20.

Spyracopoulos, L., A. A. Yee, and J. D. J. O'Neil. 1996. Backbone dynamics of an alamethicin in methanol and aqueous detergent solution determined by heteronuclear ^{1}H-^{15}N NMR spectroscopy. *J. Biomol. NMR*. 7:283–294.

Steinbach, P., and B. R. Brooks. 1994. New spherical-cutoff methods for long range forces in macromolecular simulation. *J. Comp. Chem.* 15: 667–683.

Thorgeirsson, T. E., C. J. Russell, D. S. King, and Y. K. Shin. 1996. Direct determination of membrane affinities of individual amino acids. *Biochemistry*. 35:1803–1809.

Tironi, I. G., P. Fontana, and W. F. van Gunsteren. 1996. A molecular dynamics simulation study of liquid carbon tetrachloride. *Mol. Sim.* 18:1–11.

Tobias, D. J., W. Mar, J. K. Blasie, and M. L. Klein. 1996. Molecular dynamics simulations of a protein on hydrophobic and hydrophilic surfaces. *Biophys. J.* 71:2933.

Weiner, M. C., and S. H. White. 1992. Structure of a fluid dioleoylphosphatidylcholine bilayer determined by joint refinement of x-ray and neutron diffraction data. III. Complete structure. *Biophys J.* 61: 434–447.

White, S., and W. C. Wimley. 1994. Peptides in lipid bilayers: structural and thermodynamic basis for partitioning and folding. *Curr. Opin. Struct. Biol.* 4:79–86.

Wiberg, K. B., and P. R. Rablen. 1993. Origin of the stability of carbon tetrafluoride: negative hyperconjugation reexamined. *J. Am. Chem. Soc.* 115:614–625.

Williams, R. W., and J. L. Weaver. 1990. Secondary structure of substance P bound to liposomes in organic solvents and in solution from Raman and CD spectroscopy. *J. Biol. Chem.* 265:2505–2513.

Wilmot, C. M., and J. M. Thorton. 1990. β-Turns and their distortions: a proposed new nomenclature. *Protein Eng.* 3:479–493.

Wimley, W. C., T. P. Creamer, and S. H. White. 1996. Solvation energies of amino acid side chains and backbone in a family of host-guest pentapeptides. *Biochemistry*. 35:5109–5124.

Wimley, W. C., and S. H. White. 1996. Experimentally determined hydrophobicity scale for proteins at membrane interfaces. *Nat. Struct. Biol.* 3:842–848.

Wong, T. C., and X. Gao. 1998. The temperature dependence and thermodynamic functions of partitioning of substance P peptides in dodecylphosphocholine micelles. *Biopolymers*. 45:395–403.

Woolf, T. B., and B. Roux. 1996. Structure, energetics, and dynamics of lipid-protein interactions: a molecular dynamics study of the gramicidin A channel in a DMPC bilayer. *Proteins: Struct., Funct., Genet.* 24: 92–114.

Woolley, G. A., and C. M. Deber. 1987. Peptides in membranes: lipid induced secondary structure of substance P. *Biopolymers*. 26: S109–S121.

Young, J. K., C. Anklin, and R. P. Hicks. 1994. NMR and molecular modeling investigations of the neuropeptide substance P in the presence of 15 mM sodium dodecyl sulfate micelles. *Biopolymers*. 34: 1449–1462.