Free-Energy Determinants of α-Helix Insertion into Lipid Bilayers

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ABSTRACT A detailed treatment is provided of the various free-energy terms that contribute to the transfer of a polyalanine α-helix from the aqueous phase into lipid bilayers. In agreement with previous work, the hydrophobic effect is found to provide the major driving force for helix insertion. However, an opposing effect of comparable magnitude is also identified and is attributed to the large free-energy penalty associated with the desolvation of peptide hydrogen bonds on transfer to the low dielectric environment of the bilayer. Lipid perturbation effects as well as the entropy loss associated with helix immobilization in the bilayer are also evaluated. Two configurations of a membrane-bound 25mer polyalanine helix were found to be lower in free energy than the isolated helix in the aqueous phase. The first corresponds to the case of vertical insertion, in which a helix terminus protrudes from each side of the bilayer. The second minimum is for the case of horizontal insertion, for which the helix is adsorbed upon the surface of the bilayer. The calculated free-energy minima are found to be in good agreement with recent measurements of related systems. Large free-energy barriers resulting from desolvation of unsatisfied hydrogen-bonding groups at the helix termini are obtained for both insertion processes. The barriers for insertion are significantly reduced if the helix termini are assumed to be "capped" through the formation of hydrogen bonds with polar sidechains. For uncapped helices, our results support recently proposed models in which helices are inserted by first adsorbing on the membrane surface and then having one terminus "swing around" so as to penetrate the bilayer.

INTRODUCTION

α-helices are the main building blocks of many integral membrane proteins. A detailed characterization of the energetics of insertion of α-helices into membranes is thus an important step in the understanding of membrane protein structure and function as well as of the translocation of polypeptides between cell compartments. We consider the physical origins as well as the magnitudes of the various free energy contributions to the transfer of α-helices from the aqueous phase to lipid bilayers.

The free-energy change associated with helix binding to bilayers has been measured in a number of systems. Melittin has been reported to have a binding free energy of −9 kcal/mol (Vogel, 1981), whereas cytochrome b_5 has a binding free energy of −11 kcal/mol (Leto and Holloway, 1979). Recently, Moll and Thompson (1994) measured the partition coefficients of polyalanines of different lengths between lipid bilayers and the aqueous phase. They found that a peptide of length 10, which when it is α-helical is long enough to span only half of the bilayer, is not inserted into the bilayer. In contrast, a 20mer of polyalanine can be inserted with a free energy of insertion of approximately −5 kcal/mol.

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Theoretical estimates of membrane insertion free energies of helices have yielded widely varying estimates. Assuming that the hydrophobic effect drives helix insertion and that the appropriate free-energy contribution can be estimated from free-energy surface area relationships (see also below), Engelman et al. (1986) estimated the free energy of insertion of a 20mer of polyalanine to be −30 kcal/mol. Jähnig (1983) used an estimate of the hydrophobic contribution that was similar to that of Engelman et al. (1986) but added the effects of two additional terms. The first accounts for the entropy loss that is due to to the immobilization of a helix in a bilayer as a consequence of insertion. The effect of peptide immobilization was estimated to be approximately 16 kcal/mol. The second effect results from the fact that the presence of a rigid inclusion in the membrane reduces the conformational freedom, and hence the entropy, of nearby lipid chains. This "lipid perturbation" effect results in a positive contribution to the free energy of transfer of the helix from the solution into the membrane. A numerical estimate of this free energy was given by Jähnig (1983) based on thermodynamic data concerning the "liquid crystal → gel" phase transition in lipid membranes. His estimate of ~2 kcal/mol is quite similar to the value obtained based on a recent molecular level model of the lipid perturbation effect (Fattal and Ben-Shaul, 1993). Moll and Thompson (1994) applied Jähnig's model to their polyalanine 20mer and obtained a binding free energy of −20 kcal/mol, over 15 kcal/mol more negative than the experimental value.

It is apparent that all available estimates yield insertion free energies that are far too negative. The least negative estimates include Jähnig's value of 16 kcal/mol for the immobilization free energy as the only free-energy term that counters the large hydrophobic driving force for helix in-

sertion. However, we argue below that this number is far too large and that the true cost of helix immobilization is only ~5 kcal/mol, a result that serves to increase the large disparity between theory and experiment. It seems clear from this discussion that there is something missing in current models.

We suggest that the problem is due mainly to an underestimate of the cost of inserting the peptide hydrogen bond into a low dielectric medium. Most published studies have used the partition coefficients of amino acids between water and different solvent phases (Wolfenden et al., 1981; Radzicka and Wolfenden, 1988) as a basis for estimating the free energy of partitioning of peptides between water and lipid bilayers. This procedure is quite reasonable, but it depends critically on the treatment of the energetics of insertion of the polypeptide backbone for which no simple models are available. This is because the backbone carbonyl and amino groups form hydrogen bonds in α-helices, whereas individual amino acids partition into organic solvents without forming hydrogen bonds. The energetic cost of transferring a hydrogen bond between water and organic solvents has been inferred from thermodynamic arguments (Klotz and Farnham, 1968; Roseman, 1988), but we argue below that the number is too small. The use of a larger value, derived from theoretical studies of water–alkane solubility (Sitkoff et al., 1996), yields theoretical estimates of the free energy of helix insertion that are in good agreement with experiment.

Our goal in this work is to develop a theoretical model that can accurately describe the energetics of insertion of α-helices into lipid bilayers. We focus on polyalanine helices so as to identify those properties that arise from the peptide backbone and are, therefore, common to all transmembrane helices. However, a larger goal is to provide energetic guidelines for the general behavior of helices in lipid bilayers. Indeed, the methodology presented below should be applicable for peptides and proteins of arbitrary amino acid sequence and shape. For the specific case of polyalanine, our results are in good agreement with the experimental determination of Moll and Thompson (1994) for the total free-energy cost of helix insertion. Our results are also consistent with recent suggestions that helices tend to be adsorbed at the water–membrane interface, with the helical axis parallel to the plane of the membrane (Jacobs and White, 1989; Milik and Skolnick, 1993). The physical basis of this finding is discussed further below.

Free-Energy Contributions

Following Jacobs and White (1989) (see also Engelman and Steitz, 1981; Jähnig, 1983), the total free-energy difference between peptides (proteins) in the membrane and in the aqueous phase (ΔG_{tot}) is decomposed into a sum of differences in electrostatic contributions (ΔG_{elc}), nonpolar contributions (ΔG_{np}), lipid perturbation effects (ΔG_{lip}), peptide immobilization effects (ΔG_{imm}), and contributions from peptide conformational changes such as random coil to α-helix transition (ΔG_{con}):

$$\Delta G_{tot} = \Delta G_{elc} + \Delta G_{np} + \Delta G_{lip} + \Delta G_{imm} + \Delta G_{con}. \quad (1)$$

We define a solvation free energy, ΔG_{solv}, given by

$$\Delta G_{solv} = \Delta G_{elc} + \Delta G_{np}. \quad (2)$$

ΔG_{solv} is the free energy of transfer of a solute from water to a bulk hydrocarbon phase. It accounts for electrostatic contributions resulting from changes in the solvent dielectric constant as well as for van der Waals and solvent structure effects, which are grouped in the nonpolar term and together define the classical hydrophobic effect. In principle, a more satisfying partition of the free energy would group all terms that are due to the change of environment of the α-helix in going from water to bilayer into a single environmental free-energy change given by $\Delta G_{solv} + \Delta G_{lip}$. This is because lipid perturbation effects have much in common with the hydrophobic effect in that both involve disruption of the solvent structure owing to the presence of solute. Nevertheless, we have preferred to define ΔG_{solv} as a separate term because it is calculated from water-to-alkane-phase transfer data, whereas ΔG_{lip} is estimated separately.

The following sections describe the calculation of each of the terms defined in Eq. 1. However, before proceeding to discuss the separate contributions to ΔG_{tot} we should note that this quantity is in fact a difference of *standard* free energies and thus depends on the choice of the standard state. The definition of the standard state dictates the units in which the "equilibrium constant" K is defined. K is given by

$$K = \exp(-\Delta G^0_{tot}/RT). \quad (3)$$

If we choose the standard state as 1 mol/liter (say, at room temperature), then ΔG^0_{tot} is the free-energy difference between the following two states: i) 1 mol of peptide per liter of lipid membrane and ii) 1 mol of peptide in solution. Then

$$K = C_{mem}/C_{sol} \quad (4)$$

is the partition coefficient of peptide between the membrane and the solution; C_{mem} denotes the molar concentration of peptide in the membrane, and C_{sol} is the peptide concentration in solution. Admittedly, inasmuch as the membrane is more a two-dimensional than a three-dimensional medium, expressing the partition coefficient by using C_{mem} may appear rather unusual. Yet, if one uses "molar" standard states, then it is also necessary to use molar concentrations. Alternatively, one can express the peptide partitioning between the membrane and solution in terms of the mole fractions of peptide in the two environments, namely, $K_X = X_{mem}/X_{sol}$. It is not difficult to show that (in the low-concentration limit) $K_X = K(\nu_L/\nu_W)$, where ν_L and ν_W denote the molar volumes of a lipid and water molecules,

respectively, so that

$$\Delta G_{tot}^{0,X} = -RT \ln K_X = \Delta G_{tot}^{0} - RT \ln (\nu_L/\nu_W). \quad (5)$$

Although the above paragraph is indeed elementary, we find this reminder appropriate because some of these basic notions have not been recognized in some previous estimates of the transfer free energy.

Electrostatic contributions

Calculations were based on a continuum model in which electrostatic contributions are obtained from finite difference solutions to the Poisson–Boltzmann equation (the FDPB method) (Honig et al., 1993; Honig and Nicholls, 1995). The helices are represented in atomic detail, with atomic radii and partial charges, defined at the coordinates of each nucleus. The charges and radii were taken from PARSE, a parameter set that was parameterized to reproduce gas-phase-to-water (Sitkoff et al., 1994) and alkane-to-water (Sitkoff et al., 1996) solvation free energies of small organic molecules. Specifically, the parameters used in this work reproduce alkane–water solvation free energies with an average absolute error of 0.21 kcal/mol and a maximum error of 1.15 kcal/mol.

In the FDPB calculations reported here, the boundary between the helices and the solvent (water, liquid alkane, or membrane) is obtained from the "molecular surface," which corresponds to the contact surface between the van der Waals surface of the helix and a solvent probe (defined here as having a 1.4-Å radius). The helices, the liquid alkane, and the lipid bilayer are assigned a dielectric constant of 2, whereas water has a dielectric constant of 80.

Note that, in principle, two probe radii should have been used: a 1.4-Å probe radius for those atoms of the helices that are in aqueous phase and a larger radius for the ones that are in the liquid alkane or lipid phase. To simplify computational implementation of the model, a water-sized probe radius was used for all solvents. Inaccuracies that result from this assumption have been corrected in the parameterization scheme developed for liquid alkanes (Sitkoff et al., 1996).

The electrostatic contribution is, in principle, obtained by solution of the Poisson–Boltzmann equation. However, ionic strength makes only a trivial contribution to the solvation free energies of polar molecules. As such, the effects of mobile ions (the Boltzmann terms) are ignored in this work.

The electrostatic free energy, G_{elc}^{r}, for a particular configuration, $\mathbf{r}$, of the helix, the bilayer, and the aqueous phase is given by

$$G_{elc}^{r} = \frac{1}{2} \sum_i q_i^r \Phi_i^r, \quad (6)$$

where q_i^r is the charge at a particular point in space and Φ_i^r is the electrostatic potential at this point, for the given configuration. Φ is the solution of the Poisson equation

$$\nabla \cdot \epsilon(\mathbf{r}) \nabla \Phi(\mathbf{r}) + 4\pi \rho^f(\mathbf{r}) = 0, \quad (7)$$

where $\epsilon(\mathbf{r})$ is the dielectric constant and $\rho^f(\mathbf{r})$ is the charge distribution in space (i.e., the source terms) created by the collection of the charges, q_i.

The Laplacian term in Eq. 7 was represented on a 128^3 cubic lattice by the finite-difference approximation. The lattice version of Eq. 7 was than solved for Φ by the quasi-Newton method (Holst, 1993). Calculations were carried out on a parallel CM-5 machine with 16 to 64 partitions, with a parallel version of the nonlinear Poisson–Boltzmann equation solver that we developed recently.

To determine the precision of the calculations for this type of system, we solved the Poisson equation (Eq. 7) by using two different algorithms: 1) the quasi-Newton method and 2) a simple annealing technique (Press et al., 1988), which we adapted recently to the parallel CM-5 supercomputer. The energies were found to be the same to within less than 0.1% when we used a pre-set convergence criterion of 10^{-4}. To test whether the resolution was fine enough, we calculated the electrostatic contribution to the free energy of transfer of an $(ala)_{25}$ α-helix from aqueous phase to liquid alkane in two different orientations on the grid: at the center of the xy plane parallel to the z axis and along the box diagonal. The variation in the calculated electrostatic free energy was less than 0.8 kcal/mol, which is sufficient for the purposes of this study.

Nonpolar contributions

The nonpolar contribution to the solvation free energy, G_{np}, was assumed to be proportional to the water-accessible surface area of the helix, A, through the expression

$$G_{np} = \gamma A + b. \quad (8)$$

We obtained the surface tension coefficient γ by fitting Eq. 8 to the transfer free energies of alkanes between water and liquid alkane (both hexane and cyclohexane). Using PARSE radii (Sitkoff et al., 1994), we found the coefficients to be $\gamma = 0.028$ kcal/mol·Å^2 and $b = -1.7$ kcal/mol (Sitkoff et al., 1996).

Equation 8 assumes that transfer free energies are proportional to surface area alone, whereas recent theoretical work, based on Flory–Huggins theory (Flory, 1941; Huggins, 1941; Hildebrand, 1950), has suggested that molar volume also contributes to solubility, yielding values of γ as high as 0.06 kcal/mol (DeYoung and Dill, 1990; Sharp et al., 1991; Nicholls et al., 1991; Chan and Dill, 1994; Kumar et al., 1995). However, because the relative magnitude of area- and volume-dependent contributions is not certain, we have preferred here to use Eq. 8, which effectively incorporates volume-dependent terms into the surface tension coefficient. In fact, volume and area are nearly proportional for chainlike or cylindrical molecules.

It is not clear a priori that a value of γ obtained from water–liquid alkane partition processes should be appropriate for membranes as well. However, there is good evidence that the two coefficients are very similar. For example, an analysis of the contribution of the myristylate group to the relative binding free energies to unilamellar vesicles of myristylated and nonmyristylated peptides also yields a value of γ of 0.028 kcal/mol·Å^2 (Buser et al., 1994). Very recently, Thorgeirsson et al. (1996) determined a similar value from the partitioning of the sidechains of the hydrophobic amino acids between water and lipid bilayers.

The total area of the helices accessible to lipids in a particular configuration was calculated with a modified Shrake–Rupley (1973) algorithm (Sridharan et al., 1992).

Lipid perturbation effects

Adding a nonpolar entity (such as transmembrane α-helix) into the membrane interferes with the conformational freedom of the lipid chains in the bilayer, resulting in an energy penalty for helix insertion into membranes. Based on a molecular model of the lipid chains, Fattal and Ben-Shaul (1993) estimated this free-energy penalty, in the limit of inclusion of infinite radius, to ~0.22 kcal/mol per angstrom of the circumference of the inclusion. Applying curvature corrections of the standard form $[1\text{-}(R_L/R)]$, where R_L = 3.3 Å (Tanford, 1980) and R = 5 Å are the radii of a lipid chain and the helix, respectively, we find $\Delta G_{lip} = 2\pi R[1 - (R_L/R)]0.22 = 2.3$ kcal/mol. This value is in a very good agreement with the previous estimate of 2 kcal/mol by Jähnig (1983) based on the free energy of the phase transition of the lipid bilayer from liquid crystalline to gel.

Helix immobilization

In bulk solution the helix, regarded as a rigid (rodlike) body, has three translational degrees of freedom of its center of mass and three rotational degrees of freedom. These include one degree of freedom around the helix axis (with rotation angle $0 \le \psi \le 2\pi$) and two degrees of freedom of the helix axis, corresponding to polar angles θ and ϕ, with θ denoting the angle between the helix axis and the z axis and θ the azimuthal angle between the helix axis projection in the xy plane and the x axis, specifically $0 \le \theta \le \pi$ and $0 \le \phi \le 2\pi$. For later reference let us assume that the membrane is parallel to the xy plane so that the z axis is along the membrane normal.

Upon insertion into the membrane, the helix retains two free translations and two free rotations, the two translations in the xy plane and the rotations ψ and ϕ. The other two degrees of freedom change their "identity": The translation in the z axis is more appropriately characterized as a small vibration of amplitude δz within the bilayer. Alternatively, like Jähnig (1983) and Finkelstein and Janin (1989), we can regard the helix simply as being confined to a length of δz. Similarly, the θ rotation of the helix in the membrane can be regarded as a librational motion of amplitude $\delta\theta$ or as a restricted rotation confined to this range.

Clearly, the confinement of the translational and rotational motion results in an entropy loss for the helix in the membrane compared with its free motion in solution. This effect is responsible for the immobilization free-energy contribution. A detailed theoretical model for estimating this contribution will be given elsewhere (Ben-Shaul et al., 1996). Here a shorter, yet rigorous, derivation will suffice.

It is important first to recognize that the immobilization results not from the restriction of the helix to the bilayer phase but rather from the fact that the helix is restricted within the bilayer phase. If the helix were free to move within the bilayer, there would be no immobilization effects. This provides the basis of our derivation, which defines the immobilization free energy relative to a helix that is immersed in the bilayer but that translates and rotates freely in all directions. When the helix is free to move within the bilayer, the entire volume of the bilayer, V_{mem}, is available. This is given by Ad, where A is the area of the bilayer and d is the bilayer thickness. When the helix is restricted to a small displacement in the z direction given by δz, the available volume, $\tilde{V}_{mem}$, is $A\delta z$. Because for a particle that is freely moving within a volume V the entropy is proportional to ln V, it follows that

$$\Delta G_{imm}^{trans} = -RT \ln\left[\frac{\tilde{V}_{mem}}{V_{mem}}\right] = -RT \ln\left[\frac{\delta z}{d}\right] \qquad (9)$$

Thus, given the value of δz, it is straightforward to obtain ΔG_{imm}^{trans}. Before providing a numerical estimate, it is instructive to relate the derivation given here to that of Jähnig (1983). To this end we derive Eq. 9 by using a slightly different approach.

Jähnig defined ΔG_{imm}^{trans} relative to the helix free in solution. Using this reference state, we can write $\Delta G_{imm}^{trans} = -RT \ln [(\tilde{V}_{mem}/\tilde{V}_{sol})]$, where $\tilde{V}_{mem}$ and $\tilde{V}_{sol}$ are the volumes available to the helix translation in the membrane and in the solution, respectively. If the center of mass of the helix could translate freely within the hydrophobic core of the membrane, then $\tilde{V}_{mem} = V_{mem} = Ad$, where V_{mem} is the real volume of the membrane core. However, as the helix is confined to a small range δz of the membrane thickness, one has $\tilde{V}_{mem} = Ad(\delta z/d)$. In the solution the volume available to free motion is the entire volume of the vessel; hence $\tilde{V}_{sol} = V_{sol}$. This yields $\delta G_{imm}^{trans} = -RT \ln(A\delta z/V_{sol})$. At this point Jähnig considered, arbitrarily, a 0.1-mM lipid solution in water, assumed that δz = 1 Å, and, using an approximated area per lipid of 50 Å^2, obtained $\Delta G_{imm}^{trans} \sim 8$ kcal/mol. This is indeed a good estimate of the free energy of transferring a (mole of) helix from 1 liter of water to a membrane composed of 0.1 mmol of lipid, but this is not the standard free energy of transfer, which is the difference between two standard states. The standard states involve the same concentration of helix in both environments. The standard state implies that V_{sol} has to be related to the

equivalent volume in the membrane, namely, $V_{sol} = Ad$, so that $\Delta G_{imm}^{trans} = -RT \ln(\delta z/d)$. For a 30-Å-thick membrane, adopting Jähnig's estimate of $\delta z = 1$ Å, we obtain $\Delta G_{imm}^{trans} \sim 2$ kcal/mol. In fact, we will show elsewhere (Ben-Shaul et al., 1996) that 1 Å is a good estimate for δz, so we will use the free-energy value of 2 kcal/mol in the discussion below.

The same procedure can be applied to the rotational degrees of freedom, replacing volumes with angular ranges. Jähnig (1983), assuming that the bound helix is confined to ~1° rotations compared with 360° for the free helix, estimated ~4 kcal/mol per immobilization of a rotational degree of freedom. Jähnig assumed that two rotational degrees of freedom are immobilized and estimated the total rotation free energy of immobilization as ~8 kcal/mol. However, inasmuch as the only restriction on the bound helix is in θ, as mentioned above, the rotational immobilization energy, calculated as $\Delta G_{imm}^{trans} = RT\, ln(180/1)$, reduces to ~3 kcal/mol. The total immobilization free-energy loss is, therefore, $\Delta G_{imm} \sim 5$ kcal/mol.

Conformational changes

Because isolated helices are generally not stable in aqueous solution, it is necessary to account for the free-energy change associated with the helix coil transition so that a proper comparison with experimental measurements can be made. Alanine is a good helix former, and indeed long polyalanine helices are stable in solution (Marqusee et al., 1989; Vila et al., 1992). Short helices are likely to be marginally stable, so we assume, as has been done in previous work, that $\Delta G_{con} = 0$.

RESULTS

Model helices and membrane

A model $(ala)_{25}$ α-helix was built and energy minimized, using 2000 conjugate gradient iterations and the CVFF force field (Hagler et al., 1974) in DISCOVER (Biosym Technologies, La Jolla, CA). It has a radius of 5 Å and a length (measured between the C_α atoms at its termini) of 37 Å. It was placed at different distances and orientations with respect to our model for the lipid bilayer. The bilayer was represented as a 30-Å slab with a dielectric constant of 2, known from a combination of thickness and capacitance measurements (Fettiplace et al., 1971; Dilger and Benz, 1985). The justification for this simple model is discussed further below.

Two representations of the helix were used. In one, all partial charges on polar atoms in the helix were included as source terms in the Poisson equation. In the other, the non-hydrogen-bonded polar atoms were treated as neutral. Specifically, the charges of the C and O atoms of the last four residues at the C terminus, and the charges of the N, H atoms of the first four residues at the N terminus, were set to zero. We refer to this helix as "capped" because it has no unsatisfied hydrogen-bond donor and acceptor at each terminus.

Free energy of transferring helices from the aqueous phase to liquid alkane

ΔG_{elc} of uncapped $(ala)_{25}$ α-helix from water to liquid alkane is calculated from the FDPB calculations to be 76.6 kcal/mol. The corresponding value for the capped helix is only 46.5 kcal/mol. The ~30-kcal/mol contribution of the uncompensated dipoles at the helix termini is due to only 16 of the 92 partially charged atoms of the helix. This reflects the much greater electrostatic cost of transferring isolated C═O and N—H groups compared with a single-hydrogen-bonded (C═O···H—N) group. ΔG_{np}, obtained from Eq. 8, is −50.9 kcal/mol. This value suggests that capped polyalanine helices would be expected to partition into liquid alkane, whereas uncapped helices would prefer to remain in the aqueous phase because of the electrostatic cost of transferring their uncompensated dipoles. ΔG_{elc} for transferring the capped helix, with its 21 hydrogen bonds, is 46.5 kcal/mol, or ~2.2 kcal/mol per hydrogen-bonded pair. The nonpolar contribution to each hydrogen-bonded pair, obtained as the sum of the contributions of each of its four atoms, is found to be only 0.1 kcal/mol, so the free energy of transfer of one hydrogen-bonded (C═O···H—N) group from the aqueous phase to liquid alkane is found to be 2.1 kcal/mol. A similar calculation predicts that unpaired CO and NH groups together favor the aqueous phase by 6.4 kcal/mol.

Energetics of helix insertion

We calculated the free energy of insertion of an $(ala)_{25}$ α-helix along two hypothetical pathways: vertical insertion, with the helix principal axis perpendicular to the membrane surface (Fig. 1 *A*) and horizontal insertion, with the helix principal axis parallel to the membrane surface (Fig. 1 *B*).

Vertical insertion of a single helix

Electrostatic nonpolar contributions and total free energies for the transfer of $(ala)_{25}$ helices between water and the lipid bilayer are shown in Fig. 2. Results are presented for capped and uncapped helices as a function of the distance h between the geometrical center of each helix and the geometrical center of the membrane. At $h = 36.15$ Å the helix terminus is just in contact with the membrane surface. At $h = 0$ the insertion is complete, and the helix termini protrude evenly from both sides of the membrane. The zero of energy for each helix was set to a configuration at which the helix is entirely in the aqueous phase (i.e., infinite helix–membrane distance).

It is evident from Fig. 2 that the electrostatic penalty for the uncapped helix initially increases as the depth of insertion into the bilayer increases. However, when the helix terminus begins to emerge from the far side of the bilayer

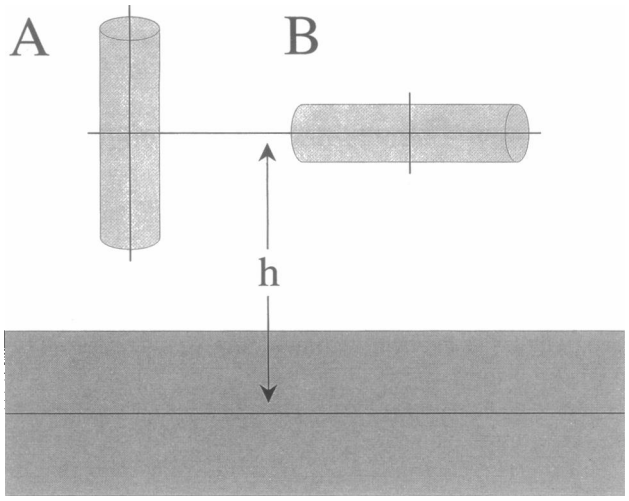

FIGURE 1 Modes of helix insertion. A schematic diagram showing two hypothetical insertion processes of an α-helix into a lipid bilayer: *A*, vertical insertion, in which the principal axis of the helix is perpendicular to the membrane surface; *B*, horizontal insertion, in which the principal axis is parallel to the membrane surface. The distance h is measured between the geometrical centers of the helix and the lipid bilayer (shaded area).

(at h = 7.5 Å), the electrostatic free energy begins to decrease until it reaches the final value of ~25 kcal/mol. For the capped helix, the electrostatic cost increases more gradually but reaches essentially the same final value (i.e., ~27 kcal/mol). This is because the uncompensated dipoles are solvated in the h = 0 configuration so that, even in the uncapped helix, only the hydrogen-bonded groups contribute to the electrostatic free energy.

The nonpolar contribution to the free energy of insertion increases until the helix begins to emerge from the other side of the bilayer, reaching a final value of ~36 kcal/mol. Thus, each fully inserted helix is predicted to be stabilized by approximately 25–36 = −11 kcal/mol relative to the isolated helix in water. However, the barrier for the insertion process is predicted to be ~12.5 kcal/mol for the uncapped helix owing to the cost of inserting the helix terminus. No barrier is found for the capped helix.

The free energy of inserting an alanine residue in helical form into the membrane is (−11/20) ~ −0.5 kcal/mol. (Only 20 alanine residues, of the total of 25 in the helix, are buried inside the membrane in the final stage of insertion.) Adopting the same strategy used above for evaluating the free energy cost of transferring a peptide backbone hydrogen bond from aqueous phase to liquid alkane, we estimate the free energy of transferring it from aqueous phase into the membrane as 1.6 kcal/mol. This is a sum of the electrostatic cost of inserting the capped helix into the membrane, ~27 kcal/mol, dividing by the 16 hydrogen bonds that are in the membrane (i.e., 1.7 kcal/mol), and adding a nonpolar contribution of −0.1 kcal/mol (as above). The number is slightly smaller than the comparable value for an alkane solvent because some of the hydrogen bonds in the helix are slightly stabilized by proximity to the aqueous phase.

Horizontal insertion

Fig. 3 plots relative solvation free energies for the case of horizontal insertion. The distance between the helix and the bilayer, h, is measured between their geometrical centers. The insertion begins at h = 22.5 Å, with the edge of the helix tangent to the membrane surface. At h = 8.0 Å the helix is fully inserted into the membrane, and at h = 0.0 Å the helix is positioned at the center of the membrane.

The electrostatic penalty for horizontal insertion is much greater than for parallel insertion because, in the former case, both termini are inserted simultaneously and never emerge from the bilayer. Regarding the total free energy, the result is, as expected, very similar to that for liquid alkane. That is, the favorable nonpolar contribution is larger than the electrostatic penalty for the capped helix, but the uncompensated dipoles in the uncapped helix ensure that full horizontal insertion will never occur.

The most significant result evident from Fig. 3 is the existence of a deep free-energy minimum (~−14 kcal/mol) near h = 19 Å, at which point the helix has been partially inserted into the bilayer. At this distance most of the helix dipoles are still fully or partially solvated, whereas there is a significant nonpolar contribution to insertion because one face of the helix is buried. That is, the nonpolar contribution increases more rapidly than for vertical insertion, because the entire helix is essentially inserted at once. As is discussed further below, the deep minimum for the partially inserted helix offers a theoretical justification for the interpretation of Jacobs and White (1989) and Yu et al (1994) of their experimental observation of the partitioning of peptides between aqueous phase and lipid bilayers.

DISCUSSION

First we discuss a number of the approximations used in this study. The description of a lipid bilayer as a low dielectric slab obscures all atomic detail about helix bilayer interactions. However, the slab model is the standard representation for the dielectric properties of the nonpolar regions of lipid bilayers and is likely to provide a reasonable model of bilayer effects on electrostatic interactions. The greatest uncertainty in the model results from its complete neglect of the polar headgroups region, which is presumably the site of helix adsorption to the bilayer. As the dielectric constant in this region is believed to be between 25 and 40 (Ashcroft et al., 1981), the polar headgroups might most appropriately be regarded as part of the aqueous phase defined in this study. This issue is be considered further below.

The calculated values for the free energy of transfer of polyalanine α-helices depends strongly on the value assigned to the inner dielectric constant and to the particular charge and radii parameter set that were used. However,

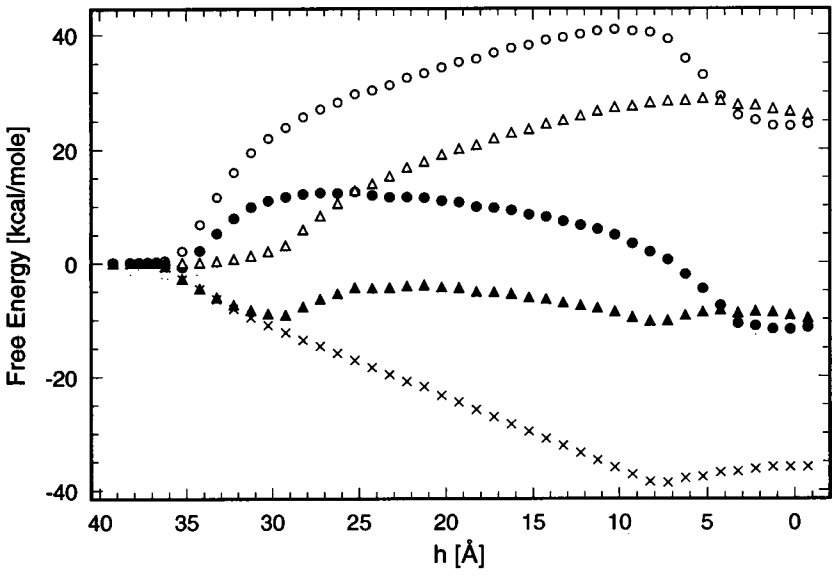

FIGURE 2 Vertical insertion. Insertion of capped and uncapped 25-polyalanine α-helices, in the orientation of Fig. 1 *A*, into a lipid bilayer. ΔG_{elc} for capped (△) and uncapped (○) helices, ΔG_{np} (×), and ΔG_{solv} for capped (▲) and uncapped (●) helices as a function of the distance h between the geometrical centers of the helix and the membrane. The zero of energy for each helix was chosen at $h = \infty$. Note that the h axis is inverted, so the insertion process proceeds from left to right.

because PARSE yields accurate transfer free energies between water and liquid alkane for amides it seems reasonable to suspect that it provides a good approximation to the water–bilayer solvation properties of alanine polypeptides that are constructed from the same chemical groups as amides. Moreover, the nonpolar surface tension contribution used in PARSE, which is deduced from the partitioning of nonpolar molecules between water and liquid alkane, is nearly identical to that reported recently for the transfer of nonpolar molecules into lipid bilayers (Buser et al., 1994; Thorgeirsson et al., 1996).

Our calculations indicate that the electrostatic free-energy penalty for transferring an uncapped α-helix from aqueous phase into liquid alkane is ~77 kcal/mol, 40% of which can be attributed to the uncompensated dipoles at the helix termini. The energy gain that is due to the nonpolar contribution is ~51 kcal/mol, suggesting that a helix with free terminal dipoles will always prefer the aqueous phase. In contrast, if the helix termini could be capped by means of hydrogen-bonding interactions with polar sidechains, the equilibrium between the two phases would shift dramatically so that, within the accuracy limits of these calculations, the helix would be expected to be present predominantly in the liquid alkane.

Two configurations of a membrane-bound helix were found to be lower in free energy than the isolated helix in the aqueous phase. The first (Fig. 2: $h = 0$ Å) corresponds to the case of complete vertical insertion in which a helix terminus protrudes from each side of the bilayer. The electrostatic free energy of this configuration is the same for the capped and the uncapped helices because the termini are fully solvated in either case. There is a free-energy penalty resulting from the dipolar groups that are buried in the bilayer. This, however, is more than compensated by the

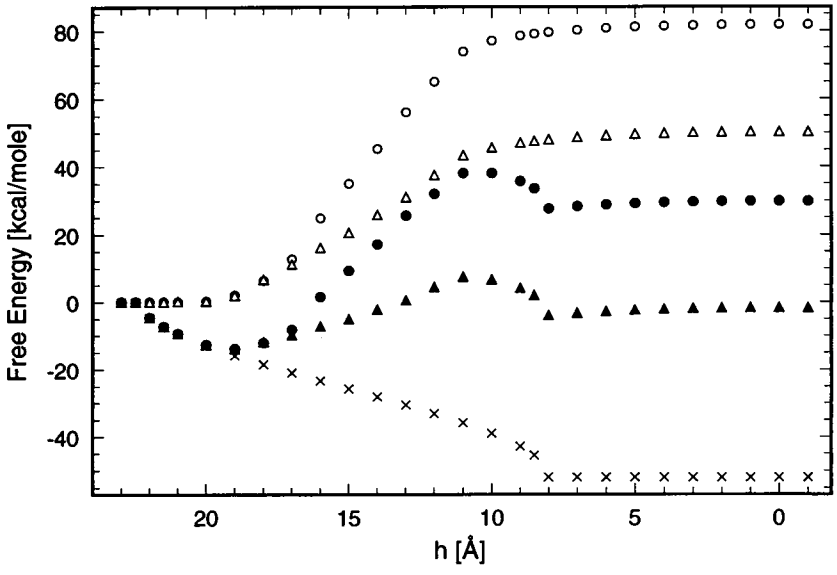

FIGURE 3 Horizontal insertion. Insertion of capped and uncapped 25-polyalanine α-helices, in the orientation of Fig. 1 *B*, into a lipid bilayer. ΔG_{elc} for capped (△) and uncapped (○) helices, ΔG_{np} (×), and ΔG_{solv} for capped (▲) and uncapped (●) helices as a function of the distance h between the geometrical centers of the helix and the membrane. The zero of energy for each helix was chosen at $h = \infty$. Note that the h axis is inverted, so the insertion process proceeds from left to right.

nonpolar contribution leading to a minimum of $\sim\Delta G_{solv} = -11$ kcal/mol for both the capped and the uncapped helices. To be able to compare this value with experimental measurements (of ΔG_{tot}), we must add estimates for all the terms defined in Eq. 1. Adding our estimates of ~2 kcal/mol for the lipid perturbation term, ΔG_{lip} and 5 kcal/mol for the immobilization free energy, ΔG_{imm}, we estimate ΔG_{tot} for vertical insertion to be ~ -4 kcal/mol.

For the case of horizontal insertion, we find that the helix adsorbed on the membrane surface (Fig. 3: $h = 19$ Å) also has a lower free energy than the free helix in aqueous solution. Here again both termini are nearly solvated, so that the electrostatic penalties for the capped and the uncapped helices are nearly the same. In this case the nonpolar contribution results from the face of the helix that interacts with the nonpolar region of the bilayer and provides the driving force for insertion. The calculated minimum relative to the aqueous phase is ~ -14 kcal/mol. There should be no lipid perturbation term for the case of horizontal insertion. To provide a rough estimate of the immobilization term we note that, in common with vertical insertion, there is a loss of one translation degree of freedom and one rotational degree of freedom. Although we have not attempted to estimate these terms explicitly for an adsorbed helix, we will assume that the estimates for vertical insertion are appropriate here as well. Adding 5 kcal/mol for ΔG_{imm} to the value of -14 kcal/mol for ΔG_{solv} yields a value of -9 kcal/mol for ΔG_{tot}.

Our estimates for water-membrane-partition free energies are thus -4 kcal/mol for vertical insertion and -9 kcal/mol for horizontal adsorption. Because we did not account for the polar headgroups region in atomic detail, and because we completely ignored the nematic field provided by the lipid phase, which would tend to favor vertical as opposed to horizontal helical orientations, the most conservative conclusion would appear to be that both configurations are approximately equally stable and that, for the case of polyalanine, both are favored relative to the isolated helix in the aqueous phase. Indeed, our free-energy estimate for the horizontally adsorbed helix is probably too negative because we did not take into account the desolvation of the polar headgroups by the helix. The theoretical values for the insertion free energy estimated here are much less negative than those reported previously and are in good agreement with the experimental range of -5 to -11 kcal/mol reported above. The theoretical value for vertical insertion of -4 kcal/mol is in excellent agreement with the value of -5.5 kcal/mol reported by Moll and Thompson (1994) for the insertion of a 20-residue polyalanine helix. (Moll and Thompson used mole fractions to calculate $\Delta G^{0,X}_{tot} = -5.5$, which converts to $\Delta G^{0}_{tot} = -3$ kcal/mol if a 1-molar standard state is used; see above.)

As was pointed out above, earlier estimates of helix insertion free energies were far more negative than those reported here and were in poor agreement with experimental values. The major difference is that, in previous work, the free-energy cost associated with transferring a hydrogen-bonded, (C=O · · · H—N) group in an α-helix was either not taken into account or assumed to be quite small. For the case of water-to-liquid-alkane transfer, our value of 2.1 kcal/mol is almost four times greater than Roseman's (1988) estimate, 0.6 kcal/mol. The latter value was obtained from a thermodynamic cycle that includes the assumption that the free energy of hydrogen-bond formation in water is 3 kcal/mol (Klotz and Farnham, 1968). This value is too high because it includes the entropic cost of dimerization, which should not be included in the intrinsic free energy of hydrogen-bond formation. A variety of theoretical calculations suggest that the free energy of hydrogen-bond formation in water is close to zero (Jorgensen, 1989; Sneddon et al., 1989; Honig and Yang, 1995; Yang and Honig, 1995). Using this value in Roseman's thermodynamic cycle yields free energy of transfer of a hydrogen bond of 3.7 kcal/mol, which is even higher than the value obtained here. Note that the free energy of transfer of a helical hydrogen bond to a lipid bilayer (1.6 kcal/mol) is calculated to be slightly less than for the transfer of this group to liquid alkane (2.1 kcal/mol) because of the partial solvation of groups near the membrane surface.

Our estimate of the free energy of transfer of an alanine residue in α-helix form from aqueous phase into the lipid bilayer is ~ -0.5 kcal/mol. This value is somewhat smaller than estimates of Jacobs and White (1989) and Milik and Skolnick (1993) of -1.5 to -2.0 kcal/mol. The difference between our estimate and theirs is that they rely on Roseman's estimate for the free energy of transfer of a hydrogen-bonded (C=O · · · H—N) group, which is less positive than ours, as mentioned above.

An important finding of this work is the existence of a deep free-energy well for the horizontal adsorption of a helix at the water–membrane interface. In this configuration, the helix termini can still be solvated, whereas a hydrophobic surface can interact with the hydrocarbon chains. This establishes strong support for the basic features of the adsorption model of Jacobs and White (1989). As shown in Fig. 3, there is a large free-energy barrier for horizontal insertion beyond the adsorbed state, as both termini must penetrate the bilayer simultaneously. This is particularly true for the uncapped helices, but even the capped helices experience a 10-kcal/mol barrier. In contrast, there is no barrier for the vertical insertion of capped helices and a much smaller, relative to the case of horizontal insertion, barrier for uncapped helices. Our results thus suggest that helices are inserted by first adsorbing in a horizontal configuration and then having one terminus "swing around" so as to penetrate the bilayer. Models of this type were proposed previously by Jacobs and White (1989) and Milik and Skolnick (1993). However, independently of the mode of insertion, an effective means of reducing the free-energy barrier is to cap the helix in some way, i.e., by using polar sidechains to satisfy as many hydrogen bonds as possible at the helix terminus.

Our results clearly indicate how amino acid sequence could control the disposition of a particular helix with respect to the lipid bilayer. Such control would be very difficult if the free-energy minima were as deep as −30 kcal/mol, as predicted by previous theories, because it would be difficult to overcome such a large driving force for helix insertion. However, our calculations point to a subtle balance between electrostatic forces that oppose helix insertion and nonpolar forces that drive insertion, leading to the shallower free-energy minima obtained in this work. Given the results for polyalanine, it is evident that specific amino acid sequences could drive the equilibrium in one direction or the other, thus offering a direct means for control of transmembrane processes. Similarly, the equilibrium between the adsorbed and transmembrane configurations could also be shifted by variations in sequence. Specifically, ringing the helix with large nonpolar sidechains would clearly favor the transmembrane state, whereas an amphiphilic helix would prefer to be adsorbed on the membrane surface. A close balance of the two effects could make the helix location dependent on additional forces, resulting, for example, from interactions with other helices or with a transmembrane potential.

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