Helix-Helix Interactions in Lipid Bilayers

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ABSTRACT Using a continuum model, we calculated the electrostatic interaction free energy between two α-helices in three environments: the aqueous phase, a low dielectric alkane phase, and a simple representation of a lipid bilayer. As was found in previous work, helix-helix interactions in the aqueous phase are quite weak, because of solvent screening, and slightly repulsive, because of desolvation effects that accompany helix assembly. In contrast, the interactions can be quite strong in a hypothetical alkane phase because desolvation effects are essentially nonexistent and because helix-helix interactions are not well screened. In this type of environment, the antiparallel helix orientation is strongly favored over the parallel orientation. In previous work we found that the free energy penalty associated with burying helix termini in a bilayer is quite high, which is why the termini tend to protrude into the solvent. Under these conditions the electrostatic interaction is strongly screened by solvent; indeed, it is sufficient for the termini to protrude a few angstroms from the two surfaces of the bilayer for their interaction to diminish almost completely. The effect is consistent with the classical model of the helix dipole in which the dipole moment is represented by point charges located at either terminus. Our results suggest, in agreement with previous models, that there is no significant nonspecific driving force for helix aggregation and, hence, that membrane protein folding must be driven by specific interactions such as close packing and salt-bridge and hydrogen bond formation.

INTRODUCTION

The transmembrane domain of most integral membrane proteins appears to consist of bundles of α-helices. The factors that drive the assembly of individual helices have been a subject of considerable interest, in part because the energetics of this process is a key element in understanding membrane protein stability. One important question concerns the extent to which there is a nonspecific driving force for helix aggregation. A possible source for this effect might be the removal of partially ordered lipid molecules from the helix surface, which would produce a lipid mediated helix-helix interaction similar in origin to the hydrophobic effect. However, calculations, based on statistical mechanical mean-field models, suggest that the effect is quite small, on the order of 1 kcal/mol per pair of interacting helices (Marcelja, 1976; Fattal and Ben-Shaul, 1993; Ben-Shaul, 1995). In this paper we consider the role of another possible source of nonspecific helix-helix interactions: the electrostatic interaction between dipoles on pairs of parallel and antiparallel α-helices. 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.

The α-helix is known to possess a substantial dipole moment which, for some applications, can be represented as two point charges of approximate magnitude of 0.5 (in atomic units) located at the helix termini (Wada, 1976). It has been suggested that the helix dipole is an important factor in a variety of protein functions (Hol, 1985) and that it can stabilize certain structural motifs in proteins (Hol et al., 1981; Hol, 1985; Sheridan et al., 1982). However, Poisson-Boltzmann (PB) calculations showed that the formation of the bundle of four α-helices in hemerythrin involves a significant loss of solvation free energy for the individual helices and only a minor gain in pairwise electrostatic stabilization, leading to the conclusion that the electrostatic helix-helix interactions oppose bundle formation in globular proteins (Gilson and Honig, 1989).

Despite the fact that electrostatic helix-helix interactions appear to play only a minor role in the aqueous phase, they might in principle be much larger in a lipid bilayer where aqueous desolvation effects are not present and where the lower dielectric constant might lead to an increase in magnitude of pairwise interactions. Indeed, it has been suggested that interactions involving the helix dipole stabilize an antiparallel arrangement of transmembrane helices (Deber and Li, 1995; Yeates et al., 1987). Our calculations do in fact suggest that electrostatic interactions between α-helices can be quite large if the helices are embedded in the low dielectric region of the bilayer. However, it is sufficient for the helix termini to protrude a few angstroms from the two surfaces of the bilayer for their interaction to be reduced significantly, close to the value obtained for helices in the aqueous phase. Our results thus suggest that helix assembly is not due to nonspecific effects, but rather must arise from quite specific interactions involving side chains on different helices. As will be discussed below, this finding is consistent with a number of recent experimental studies of helix-helix interactions in membranes.

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THEORY AND METHODS

Electrostatic free energy

Calculations are based on continuum electrostatic models that have been reviewed recently (Honig et al., 1993; Honig and Nicholls, 1995). The details are similar to the ones reported recently (Ben-Tal et al., 1996a). The helices are represented at atomic resolution, with atomic radii and partial charges defined at the coordinates of each nucleus. The appropriate parameters are taken from tables 4 and 3 of Sitkoff et al. (1994 and 1996, respectively). The boundary between the helices and the solvent (water and/or liquid alkane) is defined 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 are assigned a dielectric constant of 2. A hypothetical liquid alkane phase and the interior of a lipid bilayer are treated as a uniform medium with a dielectric constant of 2, and water is treated as a uniform medium with a dielectric constant of 80.

Electrostatic contributions to helix association are obtained from numerical solutions to the PB equation, although the effects of mobile ions (the Boltzmann terms) are ignored. The electrostatic free energy of the helices at distance r from each other, $G(r)$, is given by

$$G(r) = \frac{1}{2}\sum_i q_i(r)\phi_i(r), \quad (1)$$

where $q_i(r)$ is the charge at a particular point in space, and $\phi_i(r)$ is the electrostatic potential at this point, for the given interhelix distance r. ϕ is the solution of the Poisson equation:

$$\nabla \cdot \epsilon(\vec{r})\nabla\Phi(\vec{r}) + 4\pi\rho^f(\vec{r}) = 0, \quad (2)$$

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

The first term in Eq. 2 was represented on a 128^3 cubic lattice using the finite difference approximation. The lattice version of Eq. 2 was then solved for ϕ using the quasi-Newton method (Holst, 1993). Calculations were carried out on a parallel CM-5 machine with 16 to 64 partitions, using a parallel version of the nonlinear Poisson-Boltzmann equation solver that we developed recently.

Model helices and membrane

Three model α-helices $(ala)_n$ (n = 12, 18, and 25) were built and energy minimized in a vacuum, using 2000 conjugate gradient iterations of the CVFF forcefield (Hagler et al., 1974) in DISCOVER (MSI). The helices have a radius of 5 Å, and their lengths (measured between the C_α atoms at their termini) are 17 Å, 26 Å, and 37 Å, respectively. These helices were placed at different distances and orientations with respect to each other and with respect to our model for the lipid bilayer. The bilayer was represented as a 30-Å slab with a dielectric constant of 2. The justification for this simple model is discussed further below.

RESULTS

Helices in bulk phases

We first determine the magnitude of the electrostatic interactions between two helices in two bulk phases, water and liquid alkane (representing a pure membrane phase). The dependence of the free energy on the interhelix distance, r, measured between the geometrical centers of two parallel $(ala)_{25}$ α-helices is presented in Fig. 1 *A*, and Fig. 1 *B* summarizes the results for antiparallel helices. A strong electrostatic repulsion is observed for the parallel helices in liquid alkane, whereas a strong attraction is evident for the antiparallel helices. These interactions may be thought of as being due to the helix dipole and have been described previously (Gilson and Honig, 1989). The interactions are

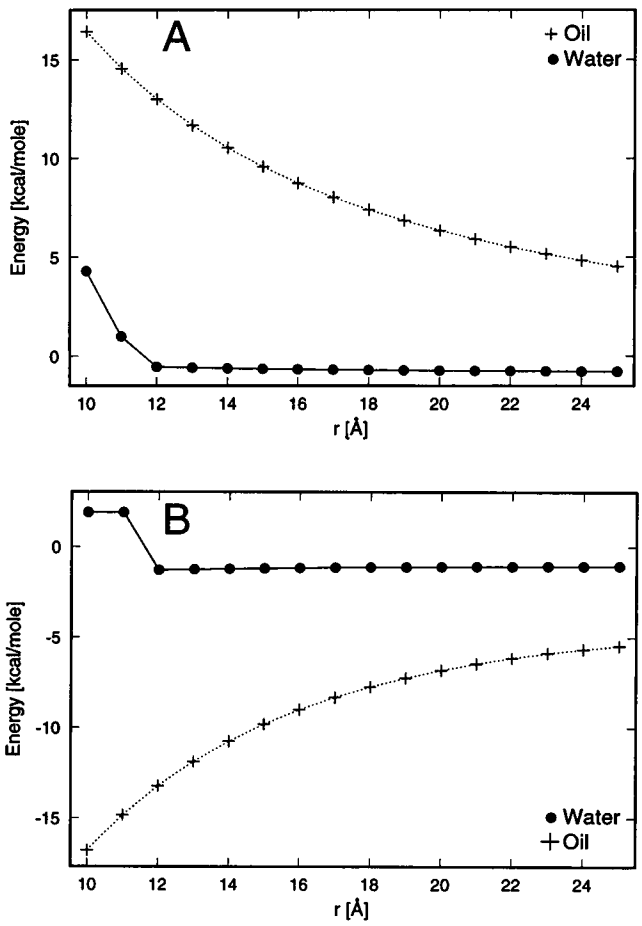

FIGURE 1 Helix-helix interactions in bulk phases. Electrostatic free energy of two (*A*) parallel and (*B*) antiparallel $(ala)_{25}$ α-helices in liquid alkane (· · ·+· · ·) and in aqueous phase (—●—) verses interhelix distance, r, measured between the geometrical centers of the helices. The zero energy for each pair of helices is at $r = \infty$.

weakened in water by a factor of 40, the ratio of the dielectric constants of the two solvents.

When the helices approach each other in water, they begin to desolvate one another, leading to an increase in electrostatic free energy. This is most evident in the antiparallel case, where the electrostatic free energy increases to about 2 kcal/mol when the helices are in contact, despite the dipolar attraction. That desolvation effects are significantly larger than pairwise interaction between the helices has been pointed out in earlier work (Gilson and Honig, 1989). This phenomenon of apparent repulsive forces between opposite charges at short distances has been noted in a number of other systems (Parsegian, 1969; Warshel, 1981; Warshel and Schlosser, 1981; Warshel and Russell, 1984; Honig and Hubbell, 1984; Novotny and Sharp, 1992; Zacharias et al., 1992; Hendsch and Tidor, 1994; Tachiya, 1994; Ben-Tal et al., 1996b). A recent survey of this phenomenon and its biological context appears in Honig and Nicholls (1995), and its physical origin was studied by Ben-Tal and Coalson (1994) and Ben-Tal (1995).

Helices in membranes

As opposed to interactions in a bulk phase, the interaction between transmembrane helices is mediated by the dielectric response of both the membrane and the aqueous phase. We have constructed polyalanine α-helices of several lengths inserted into a planar 30-Å-thick lipid bilayer (see Fig. 2). Three cases were considered: 1) $(ala)_{12}$, which is fully buried in the nonpolar phase; 2) $(ala)_{18}$, whose length is close to the thickness of the bilayer; and 3) $(ala)_{25}$, which protrudes from both sides of the bilayer.

The dependence of the electrostatic free energy on the interhelix distance r is shown in Fig. 3. As expected, the parallel helices tend to repel, and the antiparallel helices tend to attract each other. Despite their smaller dipole moment, the shortest helices experience the strongest interactions because they are entirely buried in the low dielectric regions and interact accordingly.

A particularly striking result is that the longer helices interact as if they were in the aqueous phase. This is because

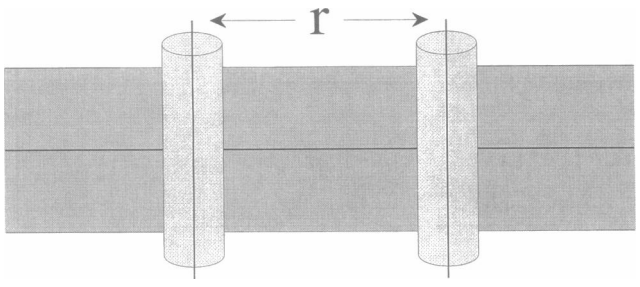

FIGURE 2 A schematic diagram of two parallel (or antiparallel) helices, separated from each other by a distance, r, measured between their geometrical centers. The helices are vertically inserted into a 30-Å-width lipid bilayer (*shaded area*) with their ends protruding out evenly from the bilayer.

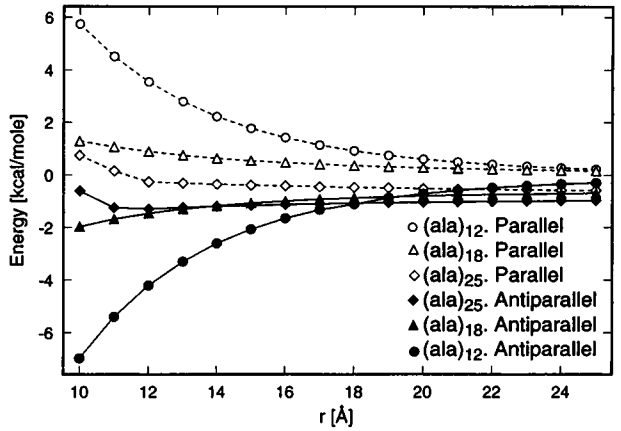

FIGURE 3 Helix-helix interactions in a lipid bilayer. Electrostatic free energy verses the interhelix distance, r, for two polyalanine α-helices. The helices are vertically inserted into a 30-Å-width lipid bilayer in the geometry of Fig. 2. · · ·○· · ·, —●—, Energies calculated for short helices of 12 residues each, parallel and antiparallel to each other, respectively. · · ·△· · ·, —▲—, Energies calculated for medium-length helices of 18 residues each, parallel and antiparallel to each other, respectively. · · ·◇· · ·, —◆—, Energies calculated for long helices of 25 residues each, parallel and antiparallel to each other, respectively. The zero energy for each pair of helices is at $r = \infty$.

the dipolar interactions can be represented as involving partial charges located at the helix termini, and these are located in the aqueous phase. On the other hand, it appears that desolvation effects are much smaller than in the aqueous phase, because much of the helix is already desolvated by virtue of its location in the bilayer. It appears from this result that there are only weak electrostatic interactions between transmembrane helices. The interaction is much stronger if the helices are fully buried in the lipid bilayer, but there is a large free energy cost associated with inserting the helix termini into the bilayer (Ben-Tal et al., 1996a).

DISCUSSION

It should first be emphasized that the description used here 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 headgroup region. Because 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. The main result of this paper is that the dipole-dipole interactions between transmembrane α-helices are quite small when the helices protrude into the aqueous phase. This result is somewhat counterintuitive because one might have expected the amide dipoles in the membrane interior to behave as if they were in a low dielectric environment and hence to interact

as strongly as totally buried helices. The source of the effect is more apparent when the helix dipole is represented as two point charges located at the termini (Wada, 1976), a model that works quite well in the aqueous phase (Gilson and Honig, 1989). Because these terminal charges are located in the aqueous phase, even though the remainder of the helix is located in the bilayer, the helix termini should be effectively screened by solvent, as is observed.

The fact that electrostatic interactions between transmembrane helices are weak, combined with the weak lipid-mediated interactions discussed above, suggests that there are no substantial nonspecific interactions that drive helix aggregation. This suggests that residue-specific interactions involving individual side chains play a central role in membrane protein folding. Many studies support this conclusion (reviewed in Bormann and Engelman, 1992; Popot, 1993; and von Heijne, 1994). Lemmon and Engelman (1992) analyzed the structure of 21 transmembrane α-helices in proteins of known structure and concluded that highly specific interactions such as interhelical salt bridges, hydrogen bonding, and precise packing are important in the folding of membrane proteins and in the oligomerization of single-helix membrane proteins. Lemmon et al. (1992) (reviewed in Cramer et al., 1992), using mutational analysis, concluded that the dimerization of glycophorin A transmembrane domain in a detergent environment depends on precise packing interactions between aliphatic amino acids. Similarly, a rotational resonance NMR study of the dimerization of the same molecule in erythrocyte membranes revealed that the dimerization is mediated by a "ridges-in-grooves" packing between two pairs of Val-Gly residues located on the dimer interface (Smith and Bormann, 1995). Deber et al. (1993) showed that Val→Ala mutations selectively alter helix-helix packing in the transmembrane segment of phage M13 coat protein, and Williams et al. (1995) showed that only small residues are tolerated at sites where transmembrane helices are in contact. Finally, Barranger-Mathys and Cafiso (1994) monitored collisions between alamethicin monomers in membranes using electron paramagnetic resonance. They concluded that alamethicin is monomeric in the absence of membrane potential, suggesting that helix aggregation can be switched on and off, based on highly specific effects induced by the membrane potential.

In previous work (Ben-Tal et al., 1996a; Ben-Shaul et al., 1996) we have found that there is a driving force on the order of 5 kcal/mol favoring the insertion of the polypeptide backbone into lipid bilayers. This force is primarily the resultant of strong hydrophobic interactions that drive helix insertion, and a strong opposing electrostatic force resulting from the desolvation of the peptide hydrogen bonds. However, if the helix termini are not fully solvated, the additional opposing electrostatic force resulting from the desolvation of unsatisfied hydrogen bond donors and acceptors (C=O and N-H groups) at the helix termini is large enough to shift the equilibrium to favor the aqueous phase. Thus the most stable orientation for a transmembrane helix is one in which both termini protrude into the aqueous phase. Under these conditions we have found here that nonspecific electrostatic interactions between the helices are essentially negligible. Thus it appears that the intrinsic driving forces for both helix insertion and helix aggregation are quite small, a feature that is necessary if the two processes are to be controlled by amino acid sequence and by factors such as the transmembrane potential.

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