Transmembrane helices of membrane proteins may flex to satisfy hydrophobic mismatch

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Abstract

A novel mechanism for membrane modulation of transmembrane protein structure, and consequently function, is suggested in which mismatch between the hydrophobic surface of the protein and the hydrophobic interior of the lipid bilayer induces a flexing or bending of a transmembrane segment of the protein. Studies on model hydrophobic transmembrane peptides predict that helices tilt to submerge the hydrophobic surface within the lipid bilayer to satisfy the hydrophobic effect if the helix length exceeds the bilayer width. The hydrophobic surface of transmembrane helix 1 (TM1) of lactose permease, LacY, is accessible to the bilayer, and too long to be accommodated in the hydrophobic portion of a typical lipid bilayer if oriented perpendicular to the membrane surface. Hence, nuclear magnetic resonance (NMR) data and molecular dynamics simulations show that TM1 from LacY may flex as well as tilt to satisfy the hydrophobic mismatch with the bilayer. In an analogous study of the hydrophobic mismatch of TM7 of bovine rhodopsin, similar flexing of the transmembrane segment near the conserved NPxxY sequence is observed. As a control, NMR data on TM5 of lacY, which is much shorter than TM1, show that TM5 is likely to tilt, but not flex, consistent with the close match between the extent of hydrophobic surface of the peptide and the hydrophobic thickness of the bilayer. These data suggest mechanisms by which the lipid bilayer in which the protein is embedded modulates conformation, and thus function, of integral membrane proteins through interactions with the hydrophobic transmembrane helices.

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1. Introduction

Accommodating the conformation of a transmembrane protein to the lipid bilayer is crucial to the structure, function and dynamics of the membrane protein [1]. A mismatch between the length of the hydrophobic surface of the transmembrane domain and the thickness of the hydrophobic interior of the membrane bilayer is expected for long hydrophobic transmembrane helices. Exposure of some of the hydrophobic surface of the helix to the aqueous environment is thermodynamically unfavorable [2] and some modification in either the structure of the protein or the structure of the bilayer or both is required.

Two models have been advanced to accommodate such a mismatch. In one model, the bilayer distorts to match the bilayer thickness with the hydrophobic surface of the transmembrane protein [3,4] and in another model, long hydrophobic helices tilt (with respect to the bilayer normal) to bury the hydrophobic surface within the hydrophobic interior of the membrane [5–9]. Although some tilted helices are observed in the few available X-ray crystal structures of membrane proteins, recent work on model helices in fluid bilayers suggested that the effect of hydrophobic mismatch on helix tilt is modest for single helices in bilayers [10]. More recently experimental and molecular dynamics evidence indicates that helix tilt in bilayers may be quite substantial [11,12]. How transmembrane protein fragments accommodate to bilayer thickness, therefore, deserves further investigation, with implications for protein folding, membrane insertion, conformation stability and functional modulation controlled through lipid interactions.

LacY is a β-galactoside transport system of E. coli encoded in the lac operon. The first report of this activity was in 1955 [13]. Subsequently, it was shown that this transport activity was
driven by a proton gradient [14], consistent with the Mitchell hypothesis [15]. This protein was cloned [16] and sequenced [17] and was purified and reconstituted in defined lipid bilayers and found to exhibit the same transport activity that had been measured in the native membrane [18]. The crystal structure shows 12 transmembrane helices, all of which are predicted to have at least some contact with the lipid [19]. The protein functions as a monomer in the membrane [20,21].

Here, experimental nuclear magnetic resonance (NMR) data on the structure, and molecular dynamics calculations on the behavior of two transmembrane segments from lacY are reported, and the results are compared with the behavior of a transmembrane segment from another transmembrane protein, bovine rhodopsin. These data suggest that the transmembrane helices of integral membrane proteins may flex as well as tilt to submerge their hydrophobic surfaces within the membrane lipid hydrocarbon core. These data also suggest that transmembrane segments of some integral membrane proteins may be more disordered than previously thought with respect to both orientation and secondary structure.

2. Materials and methods

2.1. Peptide synthesis

The following peptides were synthesized by solid phase synthesis at Genemed (San Francisco, CA):

KNTNWFGLFFYYFIMAYFPFFPIWLD Helix1 TM1
FGRAFPGVCWALCASIVMFTHNQQ Helix 5 TM5

The residues underlined were enriched in $^{15}$N synthetically in one pre-parenthesis. These peptides were analyzed by mass spectrometry and showed the correct mass for the sequence and purity greater than 90%, which is adequate for the NMR measurements. The residues in parentheses in TM5 were in the peptide studied by NMR, but not in the sequence used for molecular dynamics.

2.2. NMR spectroscopy

All NMR spectra were recorded on a Varian INOVA-600 spectrometer at 30 °C in DMSO. DMSO was used because these peptides are insoluble in water. DMSO does not preferentially stabilize any particular secondary structure, and structures of fragments in DMSO from other integral membrane proteins agreed well with corresponding regions of the X-ray crystal structures [22,23]. Standard pulse sequences and phase cycling were employed to record: double quantum filtered (DQF) COSY, and NOESY (data were collected with 400 ms mixing times [24]). Previous work with other similar-sized peptides at mixing times of 5 ps [25]. For the $^{15}$N-labeled sample, $^{15}$N-edited NOESY data were also collected. All spectra were accumulated in a phase sensitive manner using time-proportional phase incrementation for quadrature detection in F1. Chemical shifts were referenced to the residual protons in the d$_6$-DMSO.

2.3. Structure refinement

The sequence-specific assignment of the $^1$H NMR spectrum for each peptide was carried out using standard methods employing FELIX (MSI, Inc.). Assigned NOE cross peaks were segmented using a statistical segmentation function and characterized as strong, medium, and weak corresponding to upper bound distance constraints of 2.7, 3.5 and 5.0 Å, respectively. Lower bounds between non-bonded atoms were set to the sum of their van der Waals radii (approximately 1.8 Å). Pseudoatom corrections were added to interpret distance restraints where necessary [26]. Distance geometry calculations were carried out using the program DYANA [27] within the SYBYL 6.6 package (Tripos Software Inc. St. Louis). First generation DYANA structures, 150 in total, were calculated. Energy refinement calculations (restrained minimizations/dynamics) were carried out on the best distance geometry structures using the SYBYL program implementing the Kollman all-atom force field. Structures were also obtained using simulated annealing on the peptides with the distance constraints obtained from the NOESY data. Simulated annealing was done with the Kollman All Atom force field and Kollman charges within Sybyl. The molecule was heated to 1000 K for 1000 fs followed by cooling to 200 K during 1500 fs. Ten consecutive cycles were calculated. These calculations were performed on a Silicon Graphics R10000 computer.

2.4. Molecular dynamics calculations

MD simulations are performed with the GROMACS v3.1.5 package, using gromos43A2 extended to improve the simulation of the lipid components for the force field [28]. Simulations are run at a temperature of 300 K and a pressure of 1 bar in an isothermal-isobaric ensemble (NPT) with periodic boundaries present. Both Berendsen temperature and pressure couplers are chosen to keep these parameters constant. The time step for the simulations is 1 fs in the case of the short 5 ps MD simulations and 2 fs for the others. A LINCS algorithm is used to maintain the geometry of the molecules. Long-range electrostatic interactions are calculated with the particle-mesh Ewald (PME) method. PME tends to slow down the computation but increase its quality since it removes any cut-off electrostatic interactions. Lennard–Jones interactions are cut off at 14 Å. The single point charge (SPC) water model [29] is used to describe the water in the simulation box.

The bilayer consists of a 1-palmitoyl-2-oleoylphosphatidyethanolamine (POPE) patch (because the host bacterial membrane contains unsaturated PE as a major lipid) including initially 288 lipids and 16337 water molecules. POPE is a liquid–crystalline bilayer under these conditions (gel-to-liquid crystal $T_s=298.6$ K; lamellar-to-hexagonal $T_s=343$ K [30]). The topology file for the POPE molecule has been previously described and is available from [31]. The protein segment was inserted into the bilayers using a method that generates a suitable cavity in the interior of the lipid-bilayer [32] based on the solvent-accessible surface of the protein used as a template, during the course of a short steered molecular dynamics simulation (SMD) of a solvated lipid membrane (500 ps SMD run). The protein–lipid equilibration was achieved through a three-stage process. First, overlapping water molecules were removed, then lipid molecules whose headgroups were located within 1.5 Å from the protein surface were removed; the protein–lipid interface was optimized by applying repulsive forces, perpendicular to the protein surface, to the remaining lipid atoms inside the volume occupied by the protein until it was emptied. The protein itself was then inserted into the bilayer. Counter-ions were added. The system was energy-minimized at each step that involves the addition or the removal of any molecular species (steepest descent algorithm). Finally, the system was equilibrated in successive short MD simulations (5runs of 200 ps each) where position restraints were applied to the protein and progressively decreased. Simulations of 10 ns and 20 ns were run on a 1-GHz G5 (1 GB RAM) using one of two processors running under Mac OS X (10.4), taking about 75 h/ns for the system as described.

3. Results

A peptide with residues 5–36 of lacY, corresponding to the first transmembrane segment (TM1) of lacY [19], was synthesized. Multi-dimensional NMR was used to determine the structure of TM1 in solution, in DMSO. Previous work with transmembrane fragments from bacteriorhodopsin and rhodopsin demonstrated that secondary structures determined by high resolution NMR in DMSO closely matched
secondary structures of the corresponding portions of the X-ray crystal structure [23,33]. Therefore structural studies in DMSO allow the application of powerful solution-state NMR techniques not possible for the whole protein, and reveal the intrinsic secondary structural propensities of the membrane protein fragment with considerable fidelity.

Because of the high incidence of phenylalanine in the protein, peptides were made in which either all the phenylalanines were labeled with \(^{15}\text{N}\), or just the 4 adjacent phenylalanines in the middle of the peptide were labeled, to aid in the assignment. A total of 78 intraresidue, 56 sequential, and 38 long-range interactions were obtained to calculate the final structure. Fig. 1 shows the distribution of the constraints obtained from the NOESY data. The density of constraints was fairly uniform except at the carboxyl terminus. The pattern of inter-residue constraints from residue 6 to residue 30 (Fig. 1) is characteristic of helical structure. The chemical shift index [34] also suggested helicity through the same sequence (data not shown). The structure was minimized using the Powell method in Sybyl6.6. Total energy after minimization was 450 Kcal/mol. The average pairwise rmsd's of the backbone was 1.72 for peptide residues 5–32. Consistent with the data, the resulting structure showed helicity throughout most of the peptide (Fig. 2). All residues lay inside acceptable regions of the Ramachandran plot.

An overlay of the solution structure on the crystal structure is shown in Fig. 2 (see also [35]). Both structures were predominantly helical. The solution structure of the peptide fragment unraveled at both ends, typical of NMR structures of peptides [23,36,37]. The solution structure showed a break in the helix near residues 24–27 (lacY sequence), just before P28, creating a bend with an angle of about 75° (105°). The crystal structure also showed a modest break in the helix near P28 with an angle of about 20° (160°). Because the bend in the solution structure of TM1 was greater than the bend observed in the crystal structure, the solution structure deviates from the crystal structure in the overlay. This observation suggested that TM1 may have a potential to bend to a greater extent than was observed in the crystal structure.

Molecular dynamics simulations were then used to examine the behavior of TM1 of lacY in a lipid bilayer. The starting structure was helix 1 from the crystal structure. This structure was inserted into a POPE bilayer since phosphatidylethanolamine is a major phospholipid of E. coli membranes [38]. When inserted.

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**Fig. 1.** (A) Connectivity plot of the \(^1\text{H}–^1\text{H}\) NOEs observed from TM1 of lacY in solution in DMSO. (B) Plot of constraints per residue; ■ sequential; □ intraresidue; ▬ long-range.
parallel to the bilayer normal, the length of the hydrophobic surface of this helix extended beyond the hydrophobic interior of the bilayer. This initial energetically unfavorable arrangement violated the hydrophobic effect and was expected to induce a change in the system. A tilt of the helix could accommodate this violation by burying more of the hydrophobic surface of the peptide. However during the first 3 ns of simulation, the peptide did not tilt, but instead flexed to bury more of the helix in the membrane. In the first 300 ps of this MD run, a bend in the helix formed near P28 and the angle of this bend continued to decrease to a minimum at about 2 ns. Fig. 3 shows the angle between the two segments of the helix (as a measure of this flexing), as a function of time. The angle decreased by more than 25° from the initial value (from the crystal structure), and fluctuated considerably over time. The angle fluctuated between limits set by the angle of the bend observed in the crystal structure and the angle of the bend observed in the solution structure of TM1 (Fig. 2). This flexing of the helix permitted the burial of the hydrophobic surface within the hydrophobic region of the bilayer (Fig. 4A).

When the MD simulation was extended to 20 ns, another accommodation to the bilayer was observed. In the timeframe of 15 to 20 ns, the simulation showed a tilt of the helix. Fig. 4B shows a snapshot of the MD run in which the helix is tilted. Because of the truncation of the MD calculation, it is not possible to determine which solution to the hydrophobic mismatch is thermodynamically favored.

As a control, a peptide corresponding to transmembrane helix 5 of lacY (TM5) was subjected to the same analysis. The peptide contained 27 residues, 19 of which formed a continuous hydrophobic surface. The solution structure from NMR data showed a straight helix for this peptide (details described elsewhere [35]), unlike TM1. As a straight helix, the hydrophobic surface of this peptide did not significantly exceed the hydrophobic width of the bilayer. A coordinate file for TM5 was extracted from the crystal structure of lacY and placed in the same POPE bilayer used for the MD simulations of TM1, and the system was subjected to molecular dynamics for 10 ns. During the simulation the secondary structure of the helix was stable. No bend was observed. However the peptide adopted an average tilt of 12° to 18° with respect to the bilayer normal during the simulation (see Fig. 4C & 5).

For further comparison, the coordinates for a segment corresponding to helix 7-helix 8 (P285-C322) were extracted from the X-ray crystal structure of bovine rhodopsin (helix 7 is transmembrane and helix 8 is perpendicular to helix 7) [39]. This segment was placed in the same POPE bilayer for MD as the previous two peptides (the rod outer segment disk membrane is rich in phosphatidylethanolamine [40]). The helix 7 segment both flexed and tilted in the bilayer as a function of the simulation time (see Fig. 4D for a snapshot of this simulation), much like TM1 of lacY. Helix 7 flexed near P303 (in the highly conserved NPxxY sequence) where a break in the helix was observed in the crystal structure. The helix 8 segment, however, was stable at the interface between the aqueous phase and the phospholipid bilayer, anchoring the
to match the polar/hydrophobic interface on the helix was observed [5]. In this model, the distortion led to an inclusion of some phospholipids as an extension of the protein structure.

The question of whether helices tilt in a bilayer was previously addressed with a series of model peptides [10]. Helical segments, with hydrophobic stretches of alanine and leucine flanked by either tryptophan or lysine, were studied by solid state NMR and found to tilt at an angle in the bilayer that was related to the bilayer thickness; more severe tilts with thinner bilayers. Tilting of these hydrophobic transmembrane helices at least partially satisfied the mismatch with the hydrophobic width of the bilayer. The maximum tilt detected in these model peptide studies was small: 11° in the most extreme case [10]. Because the observed tilt was so small, it was suggested from these data that helix tilt was unlikely a major mechanism for accommodating hydrophobic mismatch.

However, other recent model studies revealed more extreme tilts for model peptides [12]. Furthermore, recent studies on hydrophobic transmembrane segments of membrane proteins do reveal more extreme tilts of the helices. For example, Vpu of HIV appears to form a channel, and in MD simulations the transmembrane helices tilt about 15° in small bundles and less in larger bundles of transmembrane helices [41]. NMR studies supported suggestions that Vpu tilted as much as 51°, when stressed with a narrow bilayer [11]. MD simulations of the M2 protein from influenza A virus show about a 15° tilt angle [42], while EPR studies in dimyristoylphosphatidylcholine bilayers and in dioleoylphosphatidylcholine bilayers showed tilts of 37° and 33°, respectively [43]. MD from NB of influenza B suggested a tilt angle between 5 and 10° [44]. Fluorescence measurements and MD simulations on the potassium channel, KcsA, suggested tilting of individual helices to accommodate a hydrophobic mismatch [45]. These studies suggest that helix tilt is a viable mechanism for membrane proteins to accommodate to the hydrophobic width of the bilayer in which they are embedded.

Our results from one of the helical transmembrane segments from lacY were consistent with these observations. The NMR and X-ray crystal structures of TM5 showed a continuous helix throughout the length of the peptide. In the molecular dynamics

4. Discussion

Available data now suggest that a mismatch between the length of a hydrophobic transmembrane helix and the hydrophobic thickness of a phospholipid bilayer can be solved using three mechanisms: the lipid bilayer may distort to match the protein, the transmembrane helices may tilt, and as shown in this work, the transmembrane helices may flex to match the lipid bilayer.

A good example of bilayer distortion to match the protein can be seen in the molecular dynamics study of TMS4 from the KvAPK+ channel in which a substantial distortion of the bilayer

Fig. 4. Snapshots from MD simulations: (A) 2 ns into a 2.8 ns MD simulation as described in the text showing the flexing of helix 1 of lacY in a POPE bilayer; (B) 15 ns into a 20 ns MD simulation showing tilt of helix 1 of lacY in a POPE bilayer. (C) 1 ns into a 10 ns MD simulation for helix 5 of lacY in a POPE bilayer. (D) 5 ns into a 6 ns MD simulation of helix 7/8 of rhodopsin in a POPE bilayer.

Fig. 5. Analysis of the tilt angle of a 10 ns simulation of helix 5 of lacY in a POPE bilayer.
modulated by lipid content, may therefore be a means by which helix flexing occurs.

Observations from the MD calculations (de Planque, et al, data not shown). Helix 7 of bovine rhodopsin exhibited properties consistent with the orientations (instead of a single orientation), consistent with the analysis. A limitation on this analysis is the length of time for the MD run. One can conclude that tilt is possible, but not that tilt is favored since the time of the calculation is likely a limiting factor.

The data in this report suggested helix flexing as an additional mechanism to solve problems of protein-bilayer mismatch. TM1 from lacY contains a stretch of 26 hydrophobic amino acids. The hydrophobic surface of a helix from this sequence was predicted to be about 39 Å long. Therefore if this hydrophobic helix was oriented perpendicular to the bilayer surface, the entire hydrophobic surface could not be contained within the hydrophobic region of the POPE lipid bilayer (28 Å). The NMR data and the MD calculations suggested a mechanism to alleviate this hydrophobic mismatch. Where the crystal structure showed a modest break in the helix near P28, the NMR solution structure exhibited a more pronounced bend. The MD simulations in a POPE bilayer showed that the bend near P28 oscillated with time, which was manifest as a flexing of the transmembrane helix in the bilayer. Preliminary solid-state 15N NMR data from oriented bilayers with the TM1 peptide labeled with 15N in all the phenylalanines showed a distribution of orientations (instead of a single orientation), consistent with the observations from the MD calculations (de Planque, et al, data not shown). Helix 7 of bovine rhodopsin exhibited properties similar to TM1 of lacY with a break in the helix near a proline (P303) that flexed as a function of time. Proline may be a hinge around which such flexing commonly occurs [46].

These results presented here suggest that hydrophobic mismatch between a transmembrane helix and the lipid bilayer can be accommodated by a flexing of the helix, thus decreasing the effective end-to-end length of the helix and keeping the hydrophobic surface of the helix within the hydrophobic core of the bilayer. While X-ray crystallography and NMR revealed kinked helices in a static view of membrane proteins, MD simulations reveal the dynamics of the kink, showing flexing of the helix as a function of time. It is possible that, in general, helix flexing occurs in concert with helix tilt to satisfy hydrophobic mismatches in intact membrane proteins. The relative importance of helix flexing compared to helix tilting cannot yet be determined from the available data.

The lipid bilayer may therefore modulate membrane protein tertiary structure (and thus function) in response to the width of the bilayer through both tilting and flexing of the transmembrane helices. The lipid content of the bilayer controls the width of the hydrophobic portion of the bilayer: shorter hydrocarbon chains and greater unsaturation can thin the bilayer and cholesterol can thicken the bilayer [47]. Bilayer thickness, as modulated by lipid content, may therefore be a means by which membrane lipids may modulate integral membrane protein structure and function.

Some experiments on the effect of bilayer thickness on the activity of membrane-bound enzymes have been reported. The Ca2+ Mg2+ ATPase [48,49], diacylglycerol kinase [50], rhodopsin [51], the human erythrocyte hexose transporter [52] and the Na+ K+ ATPase [53] all show sensitivity of the activity of the membrane enzyme to bilayer thickness. These effects are likely modulated through structural changes in the enzyme induced by mismatch between the thickness of the hydrophobic interior of the bilayer and the corresponding hydrophobic surface on the membrane proteins. Helix flex and helix tilt can both accommodate hydrophobic mismatch and induce protein conformational changes that would be expected to alter membrane protein function. The energy of helix–helix interactions does not appear to be so large compared to the energy cost of exposure of hydrophobic helices to water which would prohibit such conformational flexibility of these transmembrane segments [54,55].

The flexible variant with TM1 of lacY may be more general for membrane proteins with proline or perhaps also with glycine or a double glycine in the middle of the transmembrane helix. For example, MD calculations on the MscL channel suggest that kinking might play a role in the accommodation of the protein to the bilayer thickness, though the dynamics of that kinking was not reported [56]. Experimental studies on single helices in bilayers, or perhaps even membrane proteins with multiple helices in a transmembrane bundle, may need to consider a mosaic of conformations and orientations as part of the analysis, rather than a single conformation and orientation. This orientational disorder would be expected to be evident in any study in which anisotropic interactions are exploited or manifest such as in oriented bilayers. For example, a recent report describes how disorder of an oriented helix D from bacteriorhodopsin in bilayers affects the recorded PISEMA solid state NMR spectra [57]; this is also observed experimentally with the whole protein [58]. The results of the present work offer a model for understanding on a molecular level such experimentally observed orientational disorder and how it may have functional implications.

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References


