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Biophysical Chemistry 86 (2000) 79–83

Biophysical Chemistry

www.elsevier.nl/locate/bpc

Relating helix tilt in a bilayer to lipid disorder: a mean-field theory

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Received 7 April 2000; accepted 17 April 2000

Abstract

We present a mean-field theory relating the helix tilt angle in a bilayer to lipid disorder. The theory provides a method to compare the rotational barriers for different helices in lipid bilayers. The results suggest that the helix tilt angle is strongly affected by both the hydrophobicity of the helix and the average lipid disorder. This leads us to point out future experiments that could shed light on lipid–protein interactions.

Keywords: Membrane protein; Helix tilt; Lipid disorder; Mean-field theory

1. Introduction

Transmembrane helical proteins and their phospholipid bilayer have been subjected to extensive theoretical [1–4], computational [5–10] and experimental [11–23] investigations. Experiments have shown that many properties are influenced by the lipid–protein interactions, including the helix tilt angle [11,12], the activity of lactose permease [11], the folding rate of bacteriorhodopsin [14], lipid disorder [15–17] and dynamics [18], as well as the bilayer thickness [19]. However, theoretical efforts have focused on either the phospholipid bilayer [1] or the general phase behavior of the lipid–protein system [2,3], and less emphasis has been placed on the protein [4]. Therefore, in this report, we present a mean-field theory on helices in a bilayer, paying special attention to the angle between the helical axis and the bilayer normal.

2. Theory

We defined our system in Fig. 1. A helix is represented as a cylinder. θ represents the tilt angle between the helical axis and the bilayer normal. The system is cylindrically symmetric, so

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0301-4622/00/$ - see front matter
PII: S0301-4622(00)00163-0

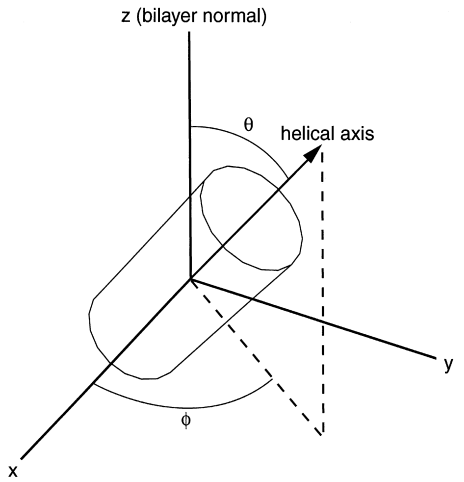

Fig. 1. Definitions of bilayer normal, helical axis, θ and ϕ. The cylinder represents the helix, and the lipid bilayer spreads along the x–y plane.

the longitudinal angle, ϕ, is unimportant. Therefore, the thermal average of the helix tilt angle is:

$$<\theta> = \left(\int_0^{\pi} \theta \exp(-H)\sin\theta d\theta\right) \Big/ \left(\int_0^{\pi} \exp(-H)\sin\theta d\theta\right) \quad (1)$$

where H is the Hamiltonian of the system, scaled by kT, with k being the Boltzmann constant and T being the absolute temperature. In analogy with a polymer in an orientation field [24], we proposed the following form for the Hamiltonian:

$$H = -\gamma < S_{\text{lipid}} > \cos\theta \quad (2)$$

where γ is a positive constant whose magnitude depends on the protein. It can be determined by fitting to experimental results, and is related qualitatively to how much energy is needed to rotate the helix in a bilayer. $< S_{\text{lipid}} >$ is the average disorder parameter of the lipid bilayer [25] that can be evaluated from NMR [25] or IR [11] spectroscopy, independently of γ.

The motivation for Eq. (2) was as follows: the cosine function ensured that $\theta = 0$ was lower in energy than $\theta = \pi$, so that it modeled the energetic cost from the disturbing of lipid packing [6] and discouraged the helix from 'flipping' across the membrane. $< S_{\text{lipid}} >$ was included because disturbing an ordered bilayer is likely to require more energy than disturbing a disordered one. As a first approximation, we used $< S_{\text{lipid}} >$ instead of the lipid disorder just around the helix because $< S_{\text{lipid}} >$ was more accessible experimentally. It is important to note that below the gel–fluid transition temperature (T_c), the bilayer may have more than one order parameter [26]. This possibility is not explicitly accounted for in Eq. (2), so we have restricted our analyses to cases where the temperature is above T_c, or to comparative studies between two peptides in the same phospholipid at similar temperatures.

Several additional comments are in order here. First, functions other than the ones in Eq. (2) could be used for lipid–protein interactions. However, all terms in Eq. (2) had clear physical motivations, and there was only one free parameter (γ) to be fitted to experiment. So, if no satisfactory fit could be found with γ being a constant, the theory is clearly deficient. Secondly, although the theory has been formulated for a single transmembrane helix, it can be used to analyze transmembrane proteins with multiple helices. We just need to look at the average tilt angle for all the helices in the same protein. However, the value obtained for γ will then be an average over all the different helices in the protein, and may not be meaningfully compared to the value obtained from samples of identical helices. Thirdly, the integration of θ in Eq. (1) accounts for the orientation entropy of the helix — something missed by calculations based solely on energy. Finally, due to the somewhat arbitrary choice for Eq. (2), the significance of γ will only be qualitative.

3. Results and discussion

We first tested our theory on lactose permease, a 12-α-helices protein that catalyses β-galactoside: H^+ symport [11]. Both $<\theta>$ and $< S_{\text{lipid}} >$ had been determined at 293 K, and various protein concentrations in a phosphatidylglycerol/phosphatidylethanolamine bilayer [11]. A con-

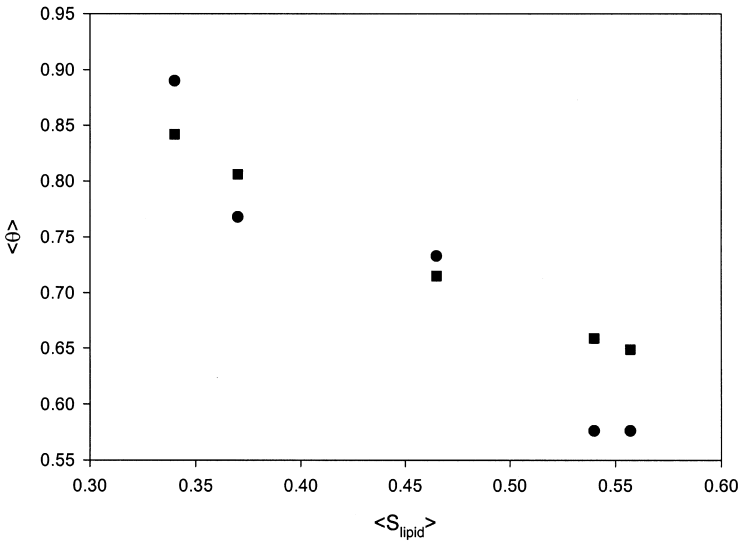

Fig. 2. Comparison between experimental and theoretical results for lactose permease. The circles (●) represent experimental results from Le Coutre et al. [11], and the squares (■) represent theoretical results from this work, with $\gamma = 7.2$ in Eq. (2).

stant γ of 7.2 in Eq. (2) gave a satisfactory agreement with the experiment (Fig. 2). This showed that Eq. (2) had captured at least the general features of lipid–helix interactions, and could be useful for other lipid–helix systems. The differences between the theoretical and experimental values showed no trend (Fig. 2), so there appeared to be no systematic error in our theory. However, the agreement deteriorated towards higher values of $< S_{\text{lipid}} >$. This could be due to interactions neglected in Eq. (2).

Next, we looked at peptides from two α-helical transmembrane ion channels: the M2-TMP peptide from influenza A virus [12]; and the M2 segment of the nicotinic acetylcholine receptor (AChR) [13]. Both were studied in dimyristoylphosphatidylcholine (DMPC) near room temperature (M2-TMP at 298 K [12]; AChR M2 at 295 K [13]); and both have 25 amino acids and form α-helices in DMPC [12,13]. Yet M2-TMP had a helical tile of $37 \pm 3°$ [12], while the tilt for AChR M2 was only approximately 12° [13]. With $< S_{\text{lipid}} > = 0.34$ [19], Eq. (2) gave $\gamma = 11.9$ and 106 for M2-TMP and AChR M2, respectively. Neither the peptide length nor the bilayer thickness could account for the difference in γ between M2-TMP and AChR M2, because they were the same for both peptides. A plausible explanation is that the more hydrophobic helix prefers to be buried in the hydrocarbon core of the bilayer, leading to a lower rotational barrier and a larger tilt angle. Our analysis showed that M2-TMP is indeed more hydrophobic than AChR M2. Using the octanol scale from White and Wimley [21], we determined the average hydrophobicity (i.e. per amino acid) of M2-TMP and AChR M2 to be -0.39 and 0.02 kcal/mol, respectively. Using a different scale, from Roseman [27], the average hydrophobicity was -0.42 kcal/mol for M2-TMP and 0.05 kcal/mol for AChR M2. These results suggested that enhancing the helix hydrophobicity increased the tilt angle. One way to test this hypothesis is to increase or reduce the hydrophobicity of M2-TMP or AChR M2 by mutation. The tilt angle of the mutant in DMPC should change accordingly. Furthermore, this could allow us to control the radius of the pore formed by the helices, so that only ions of certain sizes could pass through.

We then extended our analysis to M2-TMP in dioleoylphosphatidylcholine (DOPC) at 298 K [12]. As DOPC is approximately 4 Å thicker than DMPC, it was expected to reduce the helix tilt by 17° because of hydrophobic mismatches [12]. In-

stead, M2 showed a tilt of $33 \pm 3°$ in DOPC [12], which is almost the same as the tilt in DMPC. Kovacs et al. [12] attributed this discrepancy to specific interactions between the helices that prevent a change in the helix tilt angle. Eq. (2) suggests an additional factor in $< S_{\text{lipid}} >$. The two double bonds in DOPC should make DOPC more disordered than DMPC, as chain unsaturation generally causes disordering [28]. Furthermore, the gel–fluid transition temperature (T_c) for DMPC and DOPC was 297 and 251 K, respectively [29]. Therefore, at 298 K, $< S_{\text{lipid}} >$ would be likely to be much smaller for DOPC than for DMPC. This would lead to a smaller energetic penalty for helix rotation in the bilayer and favor a larger tilt. Therefore, the reduction in tilt from hydrophobic mismatches could have been partially neutralized by the increase in tilt from a lower value of $< S_{\text{lipid}} >$ in DOPC. This would explain the almost identical tilt in DMPC and DOPC. A simple experiment should clarify this issue: the study of the M2 protein in dipalmitoylphosphatidylcholine (DPPC) and distearoylphosphatidylcholine (DSPC). Both DPPC and DSPC are significantly longer than DMPC [19] and contain no unsaturated carbon chains, so they should reduce the helix tilt without altering $< S_{\text{lipid}} >$. However, the higher the T_c for these longer phospholipids (315 and 328 K for DPPC and DSPC, respectively [29]) must be taken into account. A possible method is to study the system at 10 K above T_c for each phospholipid, similar to [19].

Finally, we analyzed the data for gramicidin A, an ion channel protein believed to be a dimer of $\beta^{6.3}$ helices [15,19,20]. In a bilayer of dilauroylphosphatidylcholine (DLPC), the helix tilt angle was found to be $16 \pm 2°$ at 307 K [20]. From the data and Eq. (4) in de Planque et al. [19], $< S_{\text{lipid}} >$ for DLPC was calculated to be 0.41 at 283 K. So, with $< \theta > = 16°$ and $< S_{\text{lipid}} > = 0.41$, we found $\gamma = 49.5$ in Eq. (2). This value lies between those for M2-TMP ($\gamma = 11.9$) and AChR M2 ($\gamma = 106$). It seems that the $\beta^{6.3}$ helix, although more rigid than the α-helix [19], does not necessarily have a higher rotational barrier in a lipid bilayer. However, as gramicidin is significantly shorter than either M2-TMP or AChR M2 (15 vs. 25 amino acids), the γ value for gramicidin reported here may contain contributions from hydrophobic mismatches neglected in Eq. (2). Furthermore, the γ values for M2-TMP and AChR M2 have been determined for a DMPC bilayer near T_c ($= 297$ K), while the γ value for gramicidin was calculated in a DLPC bilayer above T_c ($= 273$ K). Therefore, in order to remove the ambiguity and illuminate the effect of helix conformation on the tilt angle, we propose the study of a longer $\beta^{6.3}$ helix in DMPC at 295–298 K.

4. Conclusions

The theory presented here provides a method to compare the rotational barriers for different helices in lipid bilayers. The results lead us to highlight helix hydrophobicity and the value of $< S_{\text{lipid}} >$ as possible determinants of the helix tilt angle. They also suggest that, despite a drastically different conformation, a $\beta^{6.3}$ helix may have a similar rotational barrier as an α-helix. Finally, we point out future experiments that could shed light on lipid–protein interactions.

Acknowledgements

We thank NSERC of Canada and NIH for financial support. We are also grateful to Professor Eugene I. Shakhnovich of Harvard University for encouragement and suggesting the link between hydrophobicity and the helix tilt angle.

References

[1] D.R. Fattal, A. Ben-Shaul, A molecular model for lipid–protein interactions in membranes: the role of hydrophobic mismatch, Biophys. J. 65 (1993) 1795–1808.
[2] D.A. Pink, D. Chapman, Protein–lipid interactions in bilayer membranes: a lattice model, Proc. Natl. Acad. Sci. USA 76 (1979) 1542–1546.
[3] O.G. Mouritsen, M. Bloom, Models of lipid–protein interactions in membranes, Annu. Rev. Biophys. Biomol. Struct. 22 (1993) 145–171.
[4] P.J. Park, W. Sung, Statistical mechanics of membrane protein conformation: a homopolyer model, Phys. Rev. Lett. 80 (1998) 5687–5690.

[5] K. Belochorcová, J.H. Davis, T.B. Woolf, B. Roux, Structure and dynamics of an amphiphilic peptide in a lipid bilayer: a molecular dynamics study, Biophys. J. 73 (1997) 3039–3055.
[6] M. Milik, J. Skolnick, Spontaneous insertion of polypeptide chains in membranes: a Monte Carlo model, Proc. Natl. Acad. Sci. USA 89 (1992) 9391–9395.
[7] J. Wang, A. Pullman, Do helices in membranes prefer to form bundles or stay dispersed in the lipid phase? Biochim. Biophys. Acta 1070 (1991) 493–496.
[8] A. Baumgärtner, Insertion and hairpin formation of membrane proteins: a Monte Carlo study, Biophys. J. 71 (1996) 1248–1255.
[9] L.R. Forrest, W.F. De Grado, G.R. Dieckmann, M.S.P. Sansom, Two models of the influenza A M2 channel domain: verification by comparison, Folding Des. 3 (1998) 443–448.
[10] N. Ben-Tal, B. Honig, R.M. Peitzsch, G. Denisov, S. McLaughlin, Binding of small basic peptides to membranes containing acidic lipids: theoretical models and experimental results, Biophys. J. 71 (1996) 561–575.
[11] J. Le Coutre, L.R. Narasimhan, C. Kumar, N. Patel, R. Kaback, The lipid bilayer determines helical tilt angle and function in lactose permease of *Escherichia coli*, Proc. Natl. Acad. Sci. USA 94 (1997) 10167–10171.
[12] F.A. Kovacs, J.K. Denny, Z. Song, J.R. Quine, T.A. Cross, Helix tilt of the M2 transmembrane peptide from influenza A virus: an intrinsic property, J. Mol. Biol. 295 (2000) 117–125.
[13] S.J. Opella, F.M. Marassi, J.J. Gesell, A.P. Valente, Y. Kim, M. Oblatt-Montal, M. Montal, Structures of the M2 channel-lining segments from nicotinic acetylcholine and NMDA receptors by NMR spectroscopy, Nat Struct. Biol. 6 (1999) 374–379.
[14] P.J. Booth, Folding α-helical membrane proteins: kinetic studies on bacteriorhodopsin, Folding Des. 2 (1997) R85–R92.
[15] D. Rice, E. Oldfield, Deuterium nuclear magnetic resonance studies of the interaction between dimyristoylphosphatidylcholine and gramicidin A, Biochemistry 18 (1979) 3272–3279.
[16] F. Jähnig, H. Vogel, L. Best, Unifying description of the effect of membrane proteins on lipid order. Verification for the melittin/dimyristoylphosphatidylcholine system, Biochemistry 21 (1982) 6790–6798.
[17] W. Hoffmann, D. Pink, C. Restall, D. Chapman, Intrinsic molecules in fluid phospholipid bilayers: fluorescence probe studies, Eur. J. Biochem. 114 (1981) 585–589.
[18] P. Meier, J.-H. Sachse, P.J. Brophy, D. Marsh, G. Kothe, Integral membrane proteins significantly decrease the molecular motion in lipid bilayers: a deuteron NMR relaxation study of membranes containing myelin proteolipid apoprotein, Proc. Natl. Acad. Sci. USA 84 (1987) 3704–3708.
[19] M.R.R. de Planque, D.V. Greathouse, R.E. Koeppe II, H. Schafer, D. Marsh, J.A. Killian, Influence of lipid/protein hydrophobic mismatch on the thickness of diacylphosphatidylcholine bilayers. A ^{2}H NMR and ESR study using designed tranmembrane α-helical peptides and gramicidin A, Biochemistry 37 (1998) 9333–9345.
[20] R.S. Prosser, J.H. Davis, Dynamics of an integral membrane peptide: a deuterium NMR relaxation study of gramicidin, Biophys. J. 66 (1994) 1429–1440.
[21] S.H. White, W.C. Wimley, Membrane protein folding and stability: physical principles, Annu. Rev. Biophys. Biomol. Struct. 28 (1999) 319–365.
[22] G. VonHeijne, Membrane proteins: from sequence to structure, Annu. Rev. Biophys. Biomol. Struct. 23 (1994) 167–192.
[23] C. Glaubitz, G. Gröbner, A. Watts, Structural and orientational information of the membrane embedded M13 coat protein by ^{13}C-MAS NMR spectroscopy, Biochim. Biophys. Acta 1463 (2000) 151–161.
[24] A.Y. Grosberg, A.R. Khokhlov, Statistical physics of macromolecules, American Institute of Physics Press, New York, 1994.
[25] J. Seelig, Deuterium magnetic resonance: theory and application to lipid membranes, Q. Rev. Biophys. 10 (1977) 353–418.
[26] M. Rance, K.R. Jeffrey, A.P. Tulloch, K.W. Butler, I.C.P. Smith, Orientational order of unsaturated lipids in the membranes of *Acholeplasma laidlawii* as observed by ^{2}H-NMR, Biochim. Biophys. Acta 600 (1980) 245–262.
[27] M.A. Roseman, Hydrophobicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds, J. Mol. Biol. 200 (1988) 513–522.
[28] P.F. Knowles, D. Marsh, Magnetic resonance of membranes, Biochem. J. 274 (1991) 625–641.
[29] D.B. Datta, A comprehensive introduction to membrane biochemistry, Floral Publishing, Madison, 1987.