Helix Stability and Interactions in Membrane Proteins

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Abstract

The feasibility of a qualitative separation of interaction energies between those producing stable transbilayer helices and those resulting in the side-to-side association of such helices is supported by a growing body of data. We find that the specific side-to-side interactions in a dimeric association of glycophorin A transmembrane helices can be understood in terms of a detailed packing of their van der Waals surfaces with each other. Thus, a chemical approach to membrane protein folding may succeed.

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Biol. Skr. Dan. Vid. Selsk. 1998, **49**:83–86

Introduction

Some years ago, we discussed principles of stability for helices in membranes and the use of those principles in folding and oligomerization (Engelman and Steitz, 1981; Popot and Engelman, 1990). It appears that the notion of a separation of energies into two distinct stages may have some utility in describing the ways in which helical membrane proteins fold and engage in oligomeric associations. The two-stage model for membrane protein folding (Popot and Engelman, 1990) proposes the existence of two energetically distinct events: the formation of independently stable transmembrane alpha-helices and their side-to-side association to form higher order structure (Fig. 1). Early data from bacteriorhodopsin (Popot et al., 1987) and more recent work on glycophorin (Lemmon et al., 1992; Fleming et al., 1997) generally support the ideas and have resulted in deeper insights into the chemical principles involved. However, it may be that some modifications to the model will be required; for example, it may prove to be the case that helical hairpins rather than single helices will act as stable motifs in some instances. The following is a general discussion of a current view of these ideas.

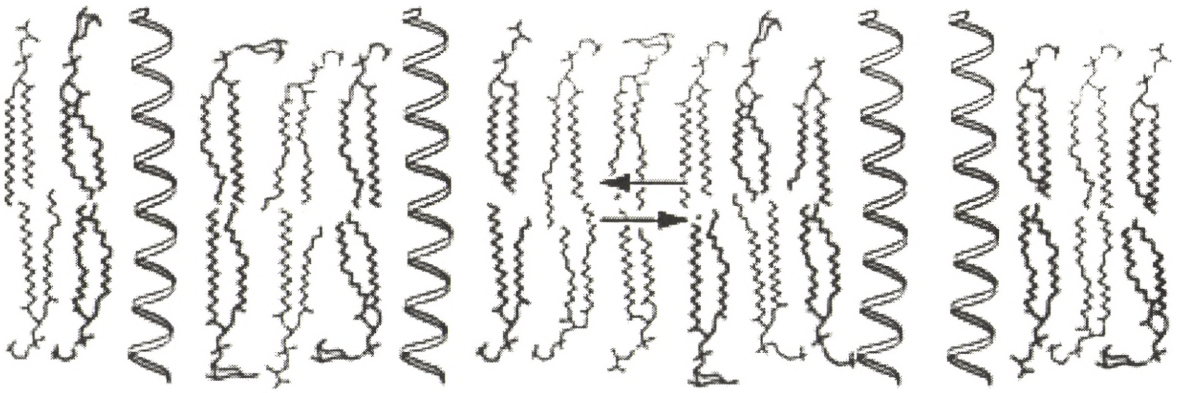

Figure 1. Two-stage model for helix association and membrane protein folding.

Helix Stability in Bilayers (Stage I)

If we consider a polypeptide having largely hydrophobic sidechains and traversing a phospholipid bilayer as a helix, we can ask which energies might contribute to its stability. It seems that two energy terms dominate in the stability of such a structure. The first is the hydrophobic effect, which favors partitioning of the helix into a hydrophobic environment and away from the aqueous environment. Based on the notion that the hydrophobic effect can be treated in terms of buried surface area, tens of kilocalories favor such a partitioning for a helix with aliphatic sidechains. The other strongly favorable energy term stabilizing the helix structure arises from the fact that the mainchain hydrogen bonds are in a low dielectric environment in which alternative donors and acceptors are absent. Thus, breaking mainchain hydrogen bonds in the hydrophobic region of the lipid bilayer carries a strong energy penalty, probably more than 5 kcal/mol per hydrogen bond. This greatly exceeds the energy corresponding to the entropy gained in a helix-coil transition, so one expects that helices will be strongly favored (or, as is well known from the porins, the alternative structure of a beta barrel may form, which also satisfies all of the hydrogen bonds). This balance of energies is supported by the observation that hydrophobicity drives helix formation even when β-branched sidechains are present (Li and Deber, 1994). Against these two stabilizing terms, and in addition to the chain entropy already mentioned, the presence of polar groups will oppose the hydrophobic effect. Some contribution of this kind is to be expected both from the mainchain of the helix and from polar sidechains. On balance, and generalizing from the widespread observation of helical structure in many membrane proteins, it appears that helical structures are strongly favored.

Helix-Helix Interactions (Stage II)

Useful views of helix-helix interactions come from studies of human glycophorin A, which forms dimers through interactions of its transmembrane helices. We have established that the individual helices are stable in the presence of sequence alterations that abolish dimerization while preserving

hydrophobicity. The fact that glycophorin dimers are stable in SDS has allowed an extensive study of sequence alterations that perturb the stability of the dimer, resulting in a large database of phenotypes for different sequences (Lemmon et al., 1992). Molecular dynamics and simulated annealing methods have been used to find side-to-side helix contacts that are favorable for the formation of dimeric structures of the glycophorin transmembrane region. Only a few favorable contacts are found; one of these agrees well with the data from sequence variation, and has led to the choice of a model for the structure (Adams et al., 1996). Using NMR spectroscopy we have now derived a structure that is independent of the previous data, and that agrees well with the model (MacKenzie et al., 1997). We have also learned a great deal more from the NMR structure concerning the details of the interaction. The fact that a largely correct model was derived from a combination of modeling and sequence variation encourages the view that reliable models for membrane proteins may be attainable without detailed crystallographic or NMR study.

Using the NMR structure, we have examined the basis of the effects of different mutations on the stability of the dimer, and we find that the dimerization is largely driven through the precise van der Waals fit of two complementary surfaces. Mutations that introduce steric clashes are strongly disruptive of the interaction, and mutations that remove van der Waals contacts also weaken the dimerization. Thus, the second stage of the two-stage model - lateral association of TM helices - can be modulated by sequence changes that affect the detailed packing between helices

We have also developed a protocol to measure the energy of interaction of the glycophorin dimer and sequence variants using analytical ultracentrifugation (Fleming et al., 1997). By using a detergent with a micelle buoyant density similar to that of buffered solvent, we observe the equilibration of the monomer and dimer of a construct of the glycophorin transmembrane domain coupled to staphylococcal nuclease. From the equilibrium distribution, we obtain equilibrium constants, and, therefore, free energies. We observe a larger proportion of dimer than was reported by the SDS assay; however, the energy changes for residue substitutions are in the same rank order of importance. Thus, it appears that the micelle environment, which is different in the two cases since different detergents were used, produces a change in the overall association constant but does not alter the hierarchy of interactions of different glycophorin molecules. If we combine this with the observation that the modeling that was successful in identifying choices for the structure is conducted *in vacuo*, we arrive at the view that the interaction of helix surfaces produces a key energy term that is not sensitive to the lipid environment. The separability of helix-helix interaction energies from the energy contributed by helix-lipid interaction would be a great simplification in understanding membrane protein folding.

Further Development of Ideas Concerning Membrane Protein Folding

While the two-stage model appears to describe much of what we have observed, it appears that some modifications may emerge. Studies of the individual helices of bacteriorhodopsin (BR) have revealed that, while the first five helices are capable of independently forming transbilayer helical structures, the last two may not be (Li and Deber, 1994), yet they are capable of reforming BR when combined with retinal and a peptide containing the first five helices (Ozawa, 1997). It may be that the link between them is necessary to stabilize a "hairpin" structure that promotes their stability as a subsection of the structure, or, possibly, that interactions with the other helices in the molecule are required to stabilize their transbilayer helical organization.

Summary

The notions of independent stability of helices and the role of helix-helix interfaces in forming membrane protein structures appear to be useful. Lipid-protein interactions, while they probably contribute to the stability of membrane proteins, may not strongly influence the details of helix interactions. If the separability of energies that we have observed is supported by further tests, it may be that good models for the transmembrane structures of helical membrane proteins can be developed on the basis of a combination of relatively limited data with molecular modeling approaches.

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