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Modest membrane hydrogen bonds deliver rich results

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Abstract

The significance of hydrogen bonds in protein structure was recognized as early as 1936 by Mirsky and Pauling, and the importance of hydrogen bonds in water-soluble proteins has since been studied extensively. Now a new paper takes an important step forward in characterizing the energetic significance of hydrogen bonds in membrane-soluble proteins.

Introduction

Hydrogen bonds are absolutely crucial for the formation of secondary structure in proteins. They also help specify tertiary structure; polar side chains in the largely hydrophobic cores of water-soluble proteins are strongly destabilizing if they do not pair with other polar groups. These key interactions generally do not contribute greatly to the overall free energy of folding. Instead, they help distinguish between alternative conformations during folding and play critical roles in substrate recognition and catalysis¹. It seems reasonable that they should play similarly important roles in membrane-soluble proteins. However, does the hydrophobic nature of the membrane change the energetics of the hydrogen bond? Joh *et al.* address this issue using mutants of the membrane protein bacteriorhodopsin². They find that the energies vary over a large range, but the average over eight such interactions is relatively modest ($-0.6 \text{ kcal mol}^{-1}$), which is in reasonable agreement with the range observed in water-soluble proteins³.

The low average contribution might seem unsettling. Indeed, many lines of evidence suggest that buried polar interactions are extremely important in membrane proteins, both biologically^{4, 5} and structurally⁶. The answer to this paradox lies in the energetic book-keeping and in the fact that when talking about folding in the membrane and folding in solution, different reference states are often assumed (Fig. 1). The unfolded states of water-soluble proteins (at least as they are generally studied *in vitro*) are generally highly solvated and largely devoid of secondary structure, thus allowing protein-water hydrogen bond formation. On the other hand, the folding of proteins inserted via the translocon involves coalescence/assembly of vertically inserted helices⁵. Thus, for most helical proteins, a natural choice of reference state is the membrane-inserted helix state, in which the side chains are exposed to the bilayer and thus hydrogen bonding with the environment is not possible. The fact that hydrogen bonds are largely satisfied in the reference state of soluble proteins, but not membrane proteins, makes the apparent contribution of hydrogen bonds to structural specificity of membrane proteins larger⁶, although the free energy contribution of the bond itself, which is what Joh *et al.* have quantified, may well be roughly the same in both environments (Fig. 1).

Until now, only a handful of studies have addressed the energetics of membrane-protein variants⁷ due to extreme difficulties associated with finding conditions in which the folded and unfolded states are truly in equilibrium and in precisely defining the unfolded state. Joh *et al.* address these difficulties by choosing to work with a well-characterized system—bacteriorhodopsin in micelles—using the established technique of a double mutant cycle. This

approach has been widely used to study the stability of water-soluble proteins⁸ because it provides an approximation of the effective folded-state interaction between two groups (coupling energy) by removing their individual free energy contributions.

Joh *et al.* find that hydrogen bond energies in the membrane are in a range similar to what is observed in water-soluble protein cores, and these findings are reminiscent of the similarities in interior composition identified by Rees and Eisenberg⁹. However, the large spread of energies measured in this work will give theorists, computational chemists and experimentalists much to sort out. For example, although the eight interactions measured here represent a very impressive piece of work, this number is insufficient to establish a correlation between the strength of a hydrogen bond and its location in the protein. We note that the hydrogen bonds that are most buried and removed from crystallographic water (Thr46-Asp96 and Thr90-Asp115 as measured using a method adapted from ref. ¹⁰) produce coupling energies that are three-fold greater than the average ($-1.7 \text{ kcal mol}^{-1}$ versus $-0.6 \text{ kcal mol}^{-1}$). Because the overall range of values is similar in water and membrane-soluble proteins, it should be possible to rationalize membrane hydrogen bond strength as it is done for water-soluble proteins—by considering the geometry, chemical nature, local dielectric and degree of burial of the interacting groups.

As with most any groundbreaking work, one can devise ways to take exception to conclusions of the Joh *et al.* study. For example, ascribing coupling energies solely to the hydrogen bonding interactions between the two residues in question rests on the assumptions that (i) only the hydrogen bond is lost upon mutating either one or both residues, (ii) no new interactions are gained and (iii) the native interaction is fully broken in the unfolded state. It is unclear whether some of the carboxyl-containing amino acids are neutral or charged under these experimental conditions, or whether they retain the same protonation states in the mutants. Finally, most of the sites studied play well-defined functional roles, so it is possible that their energetics have been evolutionarily fine-tuned (and potentially destabilized relative to the default values) to allow rapid switching between states without falling into deep energy wells.

If the core environments of membrane and soluble proteins are indeed energetically similar, the same force-field approaches extensively used for water-soluble proteins should apply in the membrane, and all that needs to be added are the proper amino acid transfer energies from solution into membrane. Furthermore, the results of Joh *et al.*, when taken in conjunction with Rees and Eisenberg's observation⁹, also suggest that membrane proteins can be converted to water-soluble proteins (and vice versa) by reversing the polarity of the exterior-facing side chains. Similar measurements to quantify the contributions of other types of protein interactions in the membrane, such as aromatic stacking, will be interesting in this context. The study by Joh *et al.* has profound implications for understanding and predicting the structures of membrane proteins, in that it provides a Rosetta stone helping to translate a large body of knowledge concerning the energetics of water-soluble proteins to the far less well understood class of membrane proteins.

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Life of polar groups destined for:

Figure 1. Energetics of potentially hydrogen bonding groups destined for membrane proteins or soluble protein cores

(a) The large cost of transferring polar groups from a solvated environment to the membrane has to be 'paid' for by the favorable burial of hydrophobic residues of the same chain. Once in the membrane, the polar group may gain some energy upon being transferred from the highly lipophilic environment of the bilayer into the protein interior. A hydrogen bond may further decrease the overall energy. (b) In solution, burial of a polar group into the hydrophobic core incurs a large energetic penalty. A core hydrogen bond often does not fully compensate for this, although cases where burial plus interaction is net favorable are known¹¹. The energy gain upon forming a hydrogen bond (last step in a and b) seems to be comparable between the membrane and the core of soluble proteins. Hydrogen bonds appear to play a more important structural role in the membrane because when membrane protein folding is discussed, it is often assumed that membrane insertion is already spontaneous, changing the reference state from solution into the membrane (reference states are marked with red).