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Similar Energetic Contributions of Packing in the Core of Membrane and Water-Soluble Proteins

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The hydrophobic effect is a major contributor to the stability of water-soluble proteins[1] but is essentially absent in the hydrocarbon core of a membrane.[2] Thus, the relative importance of other factors such as hydrogen bonding and van der Waals packing interactions must increase. While some hydrogen bonds can be key drivers of helix association,[3−5] we have argued that most appear to be modest contributors to tertiary structure stabilization in large membrane proteins.[2,6] If so, it implies that van der Waals packing dominates. Indeed, transmembrane helix dimers can be formed without any polar residues in the interface.[7]

If packing is a dominant force stabilizing membrane protein transmembrane regions, it seems possible that membrane proteins might have better optimized packing than soluble proteins. Analysis of membrane proteins leaves little consensus on this point, however. Several groups have found tighter packing in membrane proteins compared to soluble proteins.[8,9] On the other hand, Hildebrand et al. find that membrane proteins are similarly or less well packed than soluble proteins,[10] while Adamian and Liang find more cavities in membrane proteins.[11] The lack of a clear consensus on packing density differences indicates that if they exist, it remains a rather subtle effect.

Geometric analysis of static crystal structures is not always directly translatable to energetics, so we decided to probe packing contributions experimentally. Our analysis follows the classic work of Matthews and co-workers on the soluble protein T4 lysozyme.[12] In their work, the structural and energetic consequences of Leu-to-Ala substitutions in the protein core were investigated. The results are recapitulated in Figure 1. They found a remarkably linear correlation between the change in thermodynamic stability and increased cavity size, in terms of both volume and surface area, created by the core substitutions. The extrapolated free energy at zero change in cavity size (1.9 kcal mol^{-1}) provides an estimate of the change in the desolvation contribution alone (*i.e.*, due to the hydrophobic effect), without a contribution from decreased packing. The slope of the lines (24 ± 3 cal mol^{-1} Å^{-3} and 20 ± 5 cal mol^{-1} Å^{-2}) reflects the energetic cost of lost packing in the core of T4 lysozyme.

To assess packing contributions in the core of a membrane protein, we made a set of large to small substitutions at buried residues in bacteriorhopsin (V49A, L94A, L111A, I148A, I148V, and L152A) and obtained crystal structures for each of these mutants (see Table S1 in the Supporting Information). The most reliable structures were obtained for L111A (1.6 Å, R_{free} = 19.2%), L148A (2.3 Å, R_{free} = 23.6%), I148 V (1.7 Å, R_{free}= 20.6%), and L152A (1.9 Å, R_{free} = 20.2%), all from untwinned crystals. The structures

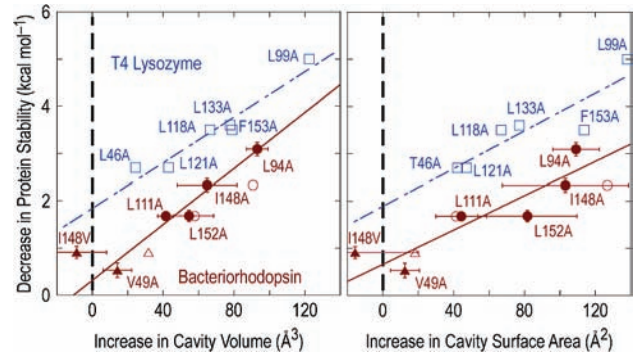

Figure 1. The energetic effects of core mutations in T4 lysozyme (blue) and bacteriorhodopsin (red) as a function of cavity size increases. Cavities were measured in terms of volume (left panel) and surface area (right panel). The data for T4 lysozyme were taken from Eriksson et al.[12] For bacteriorhodopsin, multiple conformations were observed for side chains in the newly created cavity in some cases. Filled symbols represent the cavity size using only the wild-type conformation, and the open symbols are from the weighted average using all alternate conformations. Circles indicate results for the L-to-A or I-to-A mutants that delete three carbons. Triangles indicate results for the V49A and I148 V that delete different numbers of carbons. The line was fit to only the four L-to-A or I-to-A substitutions, using the wild-type rotamers only (filled circles). Horizontal error bars indicate the standard deviation of the cavity size changes using three independent bacteriorhodopsin structures: 1C3W,[13] 1PY6,[6] and 1XJI.[14] Vertical error bars indicate the standard deviation of triplicate stability measurements. The data are provided in Tables S2−S4 in the Supporting Information.

of V49A (2.75 Å, R_{free} = 28.2%) from an untwinned crystal and L94A (2.5 Å, R_{free} = 24.5%) from a twinned crystal were of lower quality, which we were unable to improve. Nevertheless, all the crystals provided clear omit electron density maps in the area of the mutations (Figure 2). For three mutations, L111A, I148A, and I148V, removal of the side-chain atoms allowed some of the neighboring side chains (T107 and V151 for L111A; M145 for I148A and I148 V) to adopt alternate conformations that were obvious in the electron density maps (Figure 2).

We observed a clear correlation between cavity size and the change in thermodynamic stability that was measured using an SDS unfolding assay (Figure 1).[6,15] The line in the plot reflects a linear fit to the four Leu-to-Ala or Ile-to-Ala substitutions, which involve deletion of comparable size, so that the desolvation offset should be similar. The slopes of the lines for bacteriorhodopsin are 30 ± 6 cal mol^{-1} Å^{-3} and 18 ± 10 cal mol^{-1} Å^{-2}. These values are within experimental error of those found for T4 lysozyme, indicating similar packing contributions in these two proteins. When these lines are extrapolated back to zero change in cavity volume and surface area, we obtain small desolvation contributions of 0.3 ± 0.4 kcal mol^{-1} and 0.7 ± 0.9 kcal mol^{-1}, respectively. The small desolvation contribution is also indicated by the finding that the

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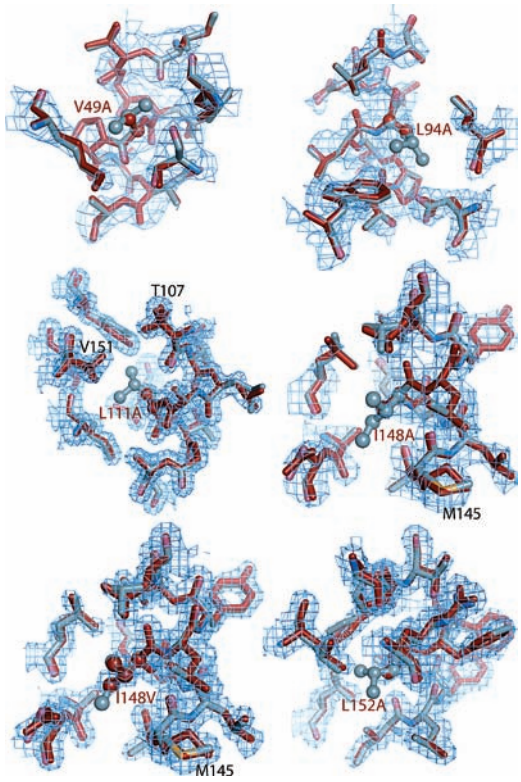

Figure 2. Comparison of the wild type and mutant bacteriorhodopsin structures. Residues within 4 Å of altered side chains are shown in stick representation, and the mutant side chain, labeled in red, is shown in ball and stick representation. The wild-type protein structure (PDB ID: 1PY6, Chain B) is shown in CPK colors, and the mutant structure is shown in red. Omit electron density is shown contoured at 1.0 σ revealing no density for the deleted atoms. The residues that adopt altered conformations in the L111A, I148A, and I148V proteins are labeled in black.

V49A and I148 V mutants fall close to the same line even though they were not included in the line fitting. Thus, any difference in the loss of desolvation due to deletions of different sizes is modest.

Our results suggest three main conclusions: (1) there is a small solvophobic effect for hydrophobic residues in detergent micelles; (2) packing of side chains makes a favorable contribution to stability indicating that they pack better in the protein core compared to the apolar chains of detergent; and (3) the energetic contribution from van der Waals packing is similar in the core of bacteriorhodopsin and T4 lysozyme.

Our results indicate that the energetic contributions of packing are similar in water and membrane soluble proteins. We cannot rule out the possibility that local regions of structure are more effectively packed than those that we probed here. For example, Eilers et al. found that the increased packing density is focused on small residues, which we cannot probe by this method.[8] It is also possible that the increased local side-chain disorder we observed for three mutants could diminish the energetic contributions we observed.

If membrane proteins do not compensate for the loss of the hydrophobic effect by increasing the energetic contributions of packing or hydrogen bonds, how might they do it? One major factor could be the reduced entropy cost of folding since the inserted membrane protein is much closer to the native state than the unfolded state of soluble proteins.[16,17] The entropy cost of folding is further reduced in membrane proteins because they bury smaller side chains on average than water-soluble proteins[8,18] with fewer rotomer choices.[19] Finally, we have observed that transmembrane regions bury more of their available surface area than soluble proteins.[20] Thus, membrane proteins may increase packing contributions not by improved packing energetics but by packing more extensively. Increasing the amount of packing may be easier for evolution to achieve than improving the packing of already well-packed protein cores.

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Supporting Information Available: Materials and methods, thermodynamic parameters for protein unfolding, X-ray diffraction data statistics, PDB codes for the mutant structures, and cavity measurements. This material is available free of charge via the Internet at http://pubs.acs.org.

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