doi:10.1016/S0022-2836(02)00920-8 available online at http://www.idealibrary.com on IDEAL® J. Mol. Biol. (2002) 323, 563–571

JMB

Standardizing the Free Energy Change of Transmembrane Helix–Helix Interactions

Karen G. Fleming

Department of Biophysics
Johns Hopkins University
3400 North Charles Street
Baltimore, MD 21218, USA

Side-to-side associations of transmembrane α-helices are integral components of the structure and function of helical membrane proteins. A fundamental unknown in the understanding of the chemical principles driving the lateral interactions between transmembrane α-helices is the balance of forces arising from the polypeptide sequence *versus* the hydrophobic solvent. To begin to address this question, a consideration of basic thermodynamic principles has been applied to assess the experimental free energy change associated with transmembrane helix dimerization in micelles. This analysis demonstrates the ability to partition the apparent free energy of transmembrane helix–helix association into two components. The first component is a statistical energy term, which arises from the fact that there are an unequal number of reactants and products. The second component is a standard state free energy change, which informs on the molecular details of the transmembrane helix self-association reaction. The advantage of separating these two energy terms arises from the fact that extrapolation to the standard state free energy change normalizes the statistical energy term so that it applies equivalently in all experimental systems. Accompanying experimental results for the glycophorin A transmembrane α-helix dimer measured in micelles are well described by these theoretical components assuming an ideal-dilute solution.

Keywords: thermodynamics; membrane protein; protein folding; α-helix association

Introduction

Genome sequencing efforts reveal that approximately one quarter of open reading frames encode proteins containing at least one transmembrane helical segment.[1] Now that potential transmembrane α-helices have been identified *en masse*, the task at hand is to solve the assembly puzzle of the lateral association of these transmembrane helices. At its core, the solution to the puzzle includes (A) establishment of the non-covalent connectivities between helices in a polytopic membrane protein as well as (B) elucidation of the propensity for hetero- and homoassociations between transmembrane helices encoded on different polypeptide chains.

These side-to-side interactions of transmembrane α-helices play a critical role in the structures, energetics and functions of helical membrane proteins. Within a polytopic membrane protein, the side-to-side interactions of transmembrane α-helices specify the arrangement of the bundle of helices that defines the native protein fold. For membrane proteins translated on different polypeptide chains, the side-to-side interactions between transmembrane α-helices play roles in mediating protein–protein associations. Functionally, such interactions may be important for cellular signaling and transport.

A consideration of the principles of stability for helices in membranes suggests that the forces influencing transmembrane helix–helix interactions will minimally include changes in protein–protein interactions, changes in lipid–lipid interactions and changes in protein–lipid interactions.[2,3] The magnitude of the free energy for each of the components is unknown. Do favorable enthalpic interactions between transmembrane helices drive their self-association strongly or does the entropy of the

Abbreviations used: ^{1}SNGpa99, a fusion protein composed of staphylococcal nuclease and the transmembrane domain of human glycophorin A; C_8E_5, pentaethylene glycol monooctyl ether.

E-mail address of the corresponding author: Karen.Fleming@jhu.edu

0022-2836/02/$ - see front matter

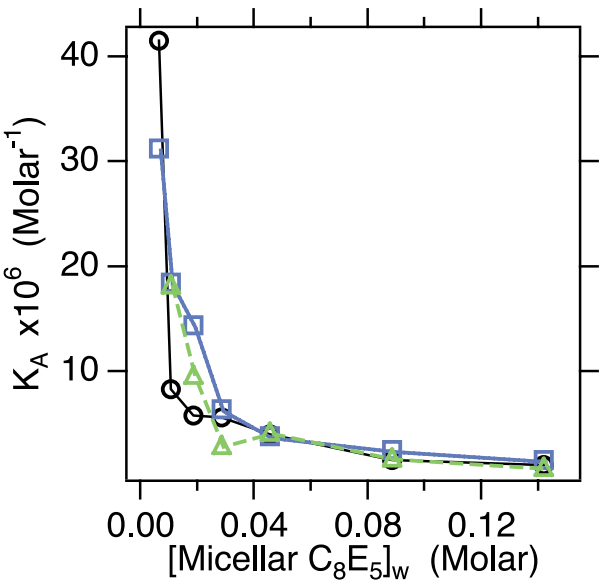

Figure 1. Variation of apparent association constant as a function of bulk aqueous C_8E_5 concentration. The experimental association equilibrium constant for the SNGpA99 transmembrane dimer (in bulk M^{-1} units) is shown as a function of the total C_8E_5 concentration (in bulk M units). Results for three independent concentration series are shown (circles, squares, and triangles), encompassing 20 sedimentation equilibrium experiments in total.

lipidic environment force transmembrane helices together? A key to understanding the lateral assembly challenge is determining where the balance lies and how each of the forces will change as a function of different lipidic environments and as a function of different protein sequences.

In recent years, several groups have made progress in experimentally measuring the free energy of transmembrane helix–helix interactions. As is the case for X-ray and NMR structural analysis of membrane proteins, the bulk of the high-resolution thermodynamic work on transmembrane helix–helix interactions has been carried out using detergent micelles as substitutes for the biological lipid bilayer. *In vitro* studies in micelles have revealed propensities for transmembrane helix–helix associations for natural sequences as well as for designed transmembrane helices.[4–15] Several biophysical techniques have been used, and many of these studies have explored the effects of changing the amino acid sequence as well as changing the micelle environment.[5,6,8,10,13,14,16] In addition, bacterial genetic assays have revealed sets of transmembrane helical sequences that associate *in vivo*.[17–23] Statistical analyses of open reading frames encoding transmembrane α-helices have recapitulated the findings of the bacterial genetic screens.[24] While these biological screens offer a more native-like environment, the stoichiometric and energetic details of the interactions discovered in the genetic assays have not been verified rigorously using biophysical tools.

One difficulty in comparing thermodynamic data between different biophysical studies and between different detergent environments arises from a lack of a clear mechanism to calculate the standard state free energy change for a given reaction in a given hydrophobic solvent environment at a given temperature. Since the protein concentration measured using analytical ultracentrifugation or resonance energy transfer is quantified spectrophotometrically, the protein concentration is most naturally obtained experimentally by relating the number of moles of protein, n_{Protein}, to the total solution volume, V_{Tot}, where V_{Tot} equals the sum of the volume of detergent micelles, V_{mic}, as well as that of the aqueous phase, V_w. For thermodynamic considerations, this concentration is inappropriate, since only the V_{mic} fraction of the total solution volume is actually available to the transmembrane protein. Thus, the effective concentration of the protein in the micellar phase is certainly very different.

To address this issue, several groups have begun to report equilibrium constants using the mole fraction scale, where the apparent equilibrium constant is normalized for the amount of detergent, e.g.:[8,9,15,16,25,26]

$$K_X = K_{A,\text{app}}\ (\text{molar}^{-1}) \times [\text{Det}]\ (\text{molar}) \qquad (1)$$

where both $K_{A,\text{app}}$ and [Det] are expressed in aqueous concentration units. The advantage of the protein–detergent mole fraction scale is that the data can be extrapolated to a common standard state, which should reveal a thermodynamically meaningful standard state free energy change (on the mole fraction scale) for the protein association reaction within the hydrophobic environment. However, for accurate calculation of this standard state free energy change, it is important to recognize that underlying the use of this equation is the premise that the protein–micellar detergent phase behaves like an ideal-dilute "solute–solvent" solution at constant chemical potential of the third solution component, water. While considerable work was carried out in the early 1900s to show that ideal solution behavior is generally true for soluble proteins at low concentrations in aqueous solutions, these assumptions have not been examined for membrane protein associations carried out in detergent micelles. Here, the model-dependent thermodynamic assumptions and equations implicit in the protein–detergent micelle mole fraction scale are explored. In addition, the theoretical considerations are tested experimentally by sedimentation equilibrium experiments carried out on the glycophorin A transmembrane domain, ^{1}SNGpA99, a molecule that has been characterized extensively as a model system for transmembrane helix–helix interactions.[4–6,10]

Results

Relating the transmembrane protein to its solvent

Using sedimentation equilibrium analysis, it is experimentally convenient and straightforward to obtain the apparent equilibrium constant for dimerization in bulk molar units, which relates the total moles of protein to the total experimental volume, V_{Tot} ($V_{mic} + V_w$; in liters) in the sedimentation equilibrium cell. Figure 1 shows the results of sedimentation equilibrium data for the GpA transmembrane dimer collected at 25 °C for seven different concentrations of detergent. These data demonstrate that the apparent association constant for protein dimerization decreases with increasing bulk concentration of detergent. At high concentrations of detergent, the data even suggest that the equilibrium constant reaches an asymptotic value. As will be shown below, such a conclusion would be incorrect. This effect is not technique-specific, since a similar trend has been observed using Forster resonance energy transfer.[8]

The concentration-dependence of the equilibrium constant raises the question of which, if any, of the experimental conditions should be used to calculate a meaningful standard state free energy of association for a given transmembrane protein sequence in a given hydrophobic environment. Investigators have skirted this question by reporting the free energy change calculated for the bulk association constant that is associated with a given concentration of detergent.[6,8] This method is clearly flawed, since this free energy value refers to an aqueous molar standard state that requires the additional specification of the cosolvent concentration. Alternatively, dissociation constant values have been reported using molar ratio[9,15] or mole fraction[8,16,25,26] units, which compare the moles of protein to the moles of detergent. However, depending on the solution behavior, a simple mole fraction calculation may or may not reveal a correct value for the standard state free energy change.

A "mass action" expression for transmembrane helix dimerization

The SNGpA99 transmembrane protein is not soluble in water alone, but requires concentrations of detergent above the critical micelle concentration for solubility. Thus, to calculate an accurate value for the free energy of dimerization, it is clear that the number of moles of protein should be related directly to the volume of its hydrophobic solvent. Concomitantly, a standard state and concentration scale must be chosen and the accompanying assumptions clarified so that the standard free energy of association can be calculated appropriately and understood. Both of these requirements can be met by consideration of the formalisms provided by the mathematics of the mass action equation for a heterogeneous equilibrium.

To address the question of transmembrane helix–helix oligomerization in micellar detergent, the thermodynamic formalisms incorporate the approximation that the micelles constitute a separate phase of matter dispersed in the aqueous phase.[27] The reaction scheme for transmembrane protein dimerization in the micellar detergent phase can then be written as:

$$2\ \text{Monomers} \leftrightarrow \text{Dimer} \tag{2}$$

The following mass action expression gives the protein association constant in the micellar detergent phase:

$$K_{EQ} = (a_{Dimer})/(a_{Monomer})^2 \tag{3}$$

where a_i represents the activity of species i. Since the concentration of detergent solvent in the micelle phase, $[Det]_{mic}$ is much greater than the concentration of the protein solute in the same phase $[P]_{mic}$, its activity is essentially constant and need not be included in the mass action expressions.

By choosing a standard state and concentration scale, one can express activities in terms of concentrations. It seems intuitive to choose the mole fraction scale for the analysis of transmembrane helix–helix associations in hydrophobic environments, since one can perceive the equilibrium constant as a protein/lipid ratio at which the equilibrium protein population is 50% monomer and 50% dimer. This choice of concentration scales may not be flawless, since it is known that the protein and detergent monomers are not of similar size. Even so, except for the volume fraction scale, it is a trivial matter to convert between the mole fraction, molar and molal concentration scales, as pointed out by White & Wimley.[2] Using the mole fraction scale, the following expression can be written:

$$K_X = (X_{Dimer})(\gamma_{Dimer})/(X_{Monomer})^2(\gamma_{Monomer})^2 \tag{4}$$

where K_X is the association equilibrium constant on the mole fraction concentration scale, and X_i and γ_i equal the concentration and activity coefficient for species i, respectively. To develop equations that test the hypothesis that the protein–micellar detergent phase behaves as an ideal-dilute solute–solvent system,[28] the activity coefficients are set to unity, which leads to:

$$K_X = (X_{Dimer})/(X_{Monomer})^2 \tag{5}$$

A numerical relationship between this thermodynamically meaningful equilibrium constant and the experimentally measured equilibrium constant obtained in the aqueous molar scale can be made by expanding the definition of the mole fraction

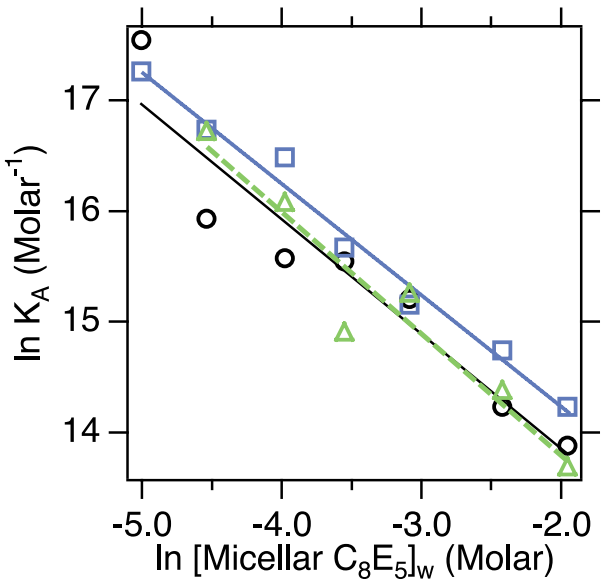

Figure 2. The transmembrane helix–C_8E_5 micellar detergent behaves as an ideal-dilute solute–solvent solution. Results for three independent concentration series (20 sedimentation equilibrium experiments in total) reveal that the ln $K_{A,app}$ varies linearly with ln[micellar $C_8E_5]_w$ with an average slope of -1.04 ($\sigma_{xn} = \pm 0.04$, $n = 3$). The linear regression of each series reveals slope values of -1.04 (circles, $R^2 = 0.90$), -1.00 (squares, $R^2 = 0.98$) and -1.09 (triangles, $R^2 = 0.92$).

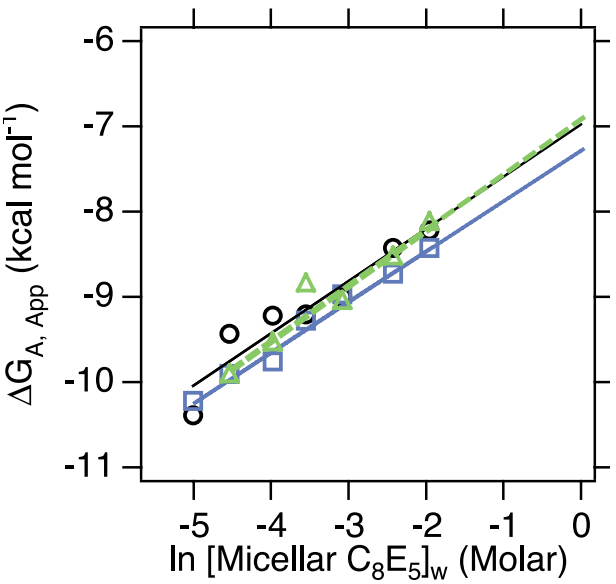

Figure 3. The mole fraction standard state free energy change for SNGpA99 transmembrane helix–helix dimerization in C_8E_5 micelles at 25 °C is -7.0 kcal mol^{-1}. Results for three independent concentration series (20 sedimentation equilibrium experiments in total) reveal that the $\Delta G_{A,app}$ varies linearly with ln[micellar $C_8E_5]_w$ with an average slope of 0.62 ($\sigma_{xn} = \pm 0.02$, $n = 3$) and an average intercept (ΔG°_X) of -7.0 kcal mol^{-1} ($\sigma_{xn-1} = \pm 0.2$, $n = 3$). The linear regression of each series reveals slope and intercept values, respectively, of 0.613 and -6.98 (circles, $R^2 = 0.90$), 0.593 and -7.24 (squares, $R^2 = 0.98$) and 0.649 and -6.87 (triangles, $R^2 = 0.92$).

variables, as shown in equation (6):

$$K_X = (X_{Dimer})/(X_{Monomer})^2$$
$$= (n_{Dimer}/n_{Total})/(n_{Monomer}/n_{Total})^2 \quad (6)$$

where the n_i equals the number of moles of species, i, in the micellar phase. As mentioned previously, the detergent concentration in the micellar phase is essentially constant and is much greater than the protein concentration, thus to a high level of accuracy, one may make the approximation that $n_{Total} \cong n_{Det}$. This approximation in equation (6) is advantageous since n_{Det} can be calculated from the bulk experimental concentration, [micellar Det]$_w$, and the total volume, V_{Tot}, employed for each experiment. By making this substitution and rearranging one arrives at equation 7:

$$K_X = (n_{Dimer}/n_{Det})/(n_{Monomer}/n_{Det})^2$$
$$= (n_{Dimer}/[\text{micellar Det}]_w V_{Total})$$
$$\div (n_{Monomer}/[\text{micellar Det}]_w V_{Total})^2$$
$$= K_{app}[\text{micellar Det}]_w \quad (7)$$

where it can be recognized that the mole fraction equilibrium constant equals the product of the concentration of micellar detergent in bulk molar aqueous units and the apparent protein equilibrium association constant in bulk M^{-1} aqueous units, which is obtained directly from fitting the sedimentation equilibrium data.

From equation (7) and if the assumptions for the ideal-dilute solution apply, it can be shown that the following relationship should be true:

$$\partial \ln K_{app}/\partial \ln [\text{micellar Det}]_w \equiv -1 \quad (8)$$

where the partial derivatives have their normal meanings of constant temperature and pressure. Figure 2 shows the experimental data presented in this manner, where it can easily be seen that the data are linear. The average of the slopes of linear regressions to three data sets in Figure 2 equals $-1.04(\pm 0.04)$, suggesting that the experimental results have been well described by the equations developed from this basic thermodynamic treatment. An experimental deviation from negative unity in the slope of this plot would be diagnostic of non-ideal behavior, which would imply the presence of a preferential interaction term that changes as a function of solvent concentration. Non-linearity of a plot on the basis of equation (8) would reflect stoichiometries other than dimer.

The standard free energy change

It follows that the standard free energy of dimerization on the mole fraction scale can be calculated from the mole fraction equilibrium constant

according to equation (7):

$$\Delta G^{\circ}_{X} = -RT \ln K_{X}$$

$$= -RT \ln(K_{app}[\text{micellar Det}]_{w}) \quad (9)$$

where ΔG°_{X} is the mole fraction standard state free energy change, R is the universal gas constant, and T is the temperature in K. Upon rearrangement, one arrives at the following expression:

$$\Delta G_{app} = \Delta G^{\circ}_{X} + RT \ln[\text{micellar Det}]_{w} \quad (10)$$

where $\Delta G_{app} = -RT \ln K_{app}$, which is the apparent free energy change obtained directly from the sedimentation equilibrium experiment. It is easily recognized from equation (10) that this experimentally observed free energy change is a linear function with a slope of RT and with the intercept equal to the mole fraction standard state free energy change. Figure 3 shows the apparent free energy values plotted as a function of $\ln$ [micellar Det]$_w$. The mole fraction standard state free energy of association for the glycophorin A transmembrane helix in C_8E_5 micelles at 25 °C was found to be -7.0 kcal mol^{-1}.

Discussion

Validation of the true standard state equilibrium constant

Membrane proteins are not soluble in aqueous solution in the absence of a hydrophobic solvent. For biophysical studies carried out *in vitro* as well as high-resolution structural studies such as NMR or X-ray crystallography, the hydrophobic solvent is often provided by detergent micelles. Application of mathematical thermodynamic formalisms outlines a clear and concise framework for consideration of energetic measurements of transmembrane helix–helix interactions in micellar environments. Of principal importance is the fact that this thermodynamic analysis shows the derivation of equation (1) from first principles on the basis of a consideration of the solution chemical potential. Thus, the validity of using equation (1) as a basis for calculation of a standard state free energy change is examined and tested.

Determination of an unequivocal value for the standard state free energy change of the reaction is an important first parameter that must be known for the subsequent dissection of transmembrane helix–helix structural energetics. In the ideal case, the apparent free energy of dimerization has an intrinsic chemical component reflecting all of the specific chemical interactions operating and a statistical energy term that applies to any reaction with unequal numbers of reactants and products. For SNGpA99 in C_8E_5 at 25 °C, the first component is a mole fraction standard state free energy of association equal to -7.0 kcal mol^{-1}. This free energy change reflects the details of the chemical and molecular reactions involved in transmembrane helix–helix association and contains contributions from changes in protein–protein interactions, changes in protein–detergent interactions, changes in detergent–detergent interactions as well as changes in molecular entropy, such as side-chain conformational entropy. The second energy term modulating the equilibrium population under any experimental condition arises from a free energy of mixing. In this ideal case of SNGpA99 in C_8E_5, this free energy of mixing originates from an entropy of mixing, also known as the cratic entropy.[29]

The advantage of distinguishing these two energy terms arises from the fact that extrapolation to the standard state free energy change is a mechanism to normalize the statistical energy term so that it applies equivalently in all experimental systems. A clear understanding of how to calculate the standard state free energy change value accurately for transmembrane helix–helix interactions in micellar detergent solutions represents an advance in our ability to compare thermodynamic data obtained in different hydrophobic environments.

Application to other detergent micelle environments

A question that arises from this experimental analysis is whether the phenomenon will be true for other micelle environments. Fisher *et al*.[8] have used fluorescence resonance energy transfer measurements to determine the dimerization equilibrium of synthetic peptides corresponding to the transmembrane domain of glycophorin A. Analysis of their data (their Table 1) is shown in Figure 4(a). A linear regression of the data revealed a slope of 0.442 kcal mol^{-1} K (small dotted line in Figure 4(a), $R^2 = 0.92$), which deviates by 25% from RT at room temperature. Because the slope is not RT, the extrapolated intercept value from the linear fit (-5.48 kcal mol^{-1}) is likely to be a numerical overestimate caused by the reduced slope. A source of slope deviation can result from a concentration-dependence of the activity coefficient(s) of any of the species, and this may be the case for SDS, since strong charges are well known to exhibit variations in activity coefficients as a function of concentration.[30] To address this possibility, equation (10) was modified to include a non-ideality term for the micellar SDS detergent, which leads to the following expression:

$$\Delta G_{app} = \Delta G^{\circ}_{X} + RT \ln([\text{micellar Det}]_{w}\gamma_{Det}) \quad (11)$$

where γ_{Det} is the non-ideality term due to the micellar SDS. Since the micellar SDS activity coefficient was not determined, an attempt was made to computationally fit for it. Sensitivity

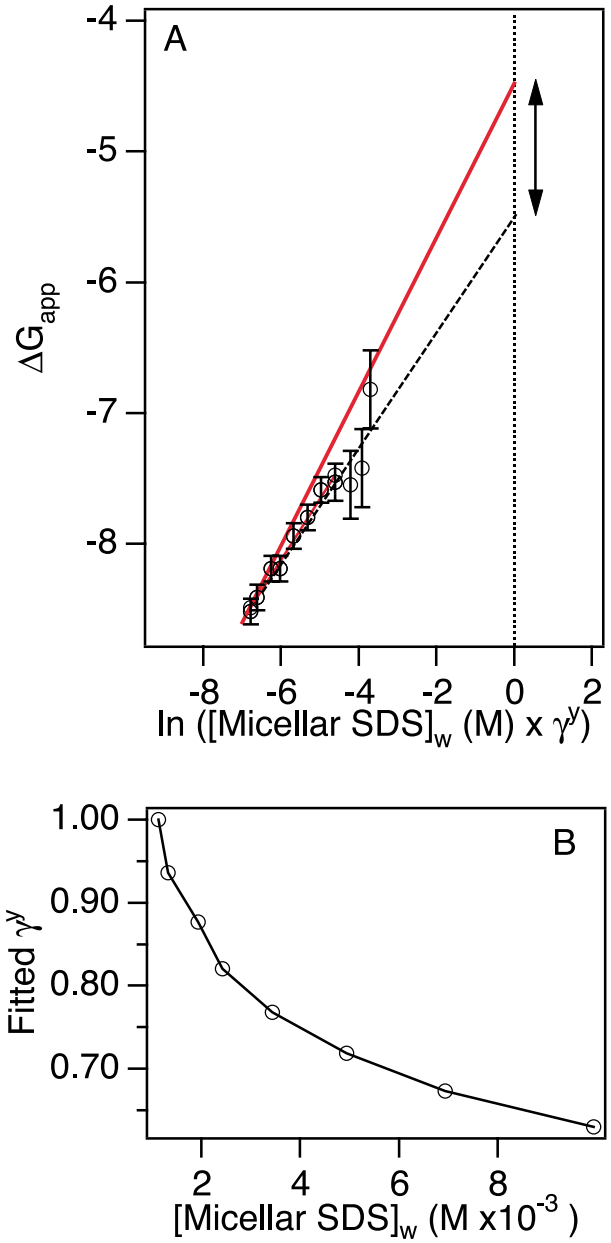

Figure 4. Analysis of GpA transmembrane dimerization in SDS micelles. Primary data used are from Fisher *et al.*[8] (Table 1); error bars are estimated from Figure 9(b) of Fisher *et al.*[8] (a) The circles represent the data. The small dotted line is a linear regression through the data. The continuous line is the calculated line using fitted activity coefficient, $\gamma = 1$ at the lowest concentration of SDS. The vertical arrow indicates the range of standard state free energies bracketed by the two extrapolations. (b) The fitted activity coefficient products are shown as a function of micellar concentration of SDS.

analysis showed that a large set of values can provide solutions to equation (11) resulting in a slope of *RT*. Assuming $\gamma = 1$ at the lowest experimental concentration of SDS, the continuous line shown in Figure 4(a) was obtained. This line yielded a standard state free energy change of -4.48 kcal mol^{-1}. The values of the fitted activity coefficients are shown in Figure 4(b), where it can be seen that the shape of the concentration-dependence curve resembles that of known activity curves for ions in solution.[30] Thus, it is likely that the standard state free energy change in SDS at 25 °C is in the range of -5.5 to -4.5 kcal mol^{-1}. These thermodynamic data in SDS reveal how a concentration series exploring the dependence of K_{app} on [micellar Det]$_w$ can be used to distinguish between ideal and non-ideal behavior. Due to the non-ideality, determination of an unequivocal standard state free energy change in SDS will require additional experimentation.

Adjustment of the equilibrium ensemble by dilution

At its core, equation (8) describes dilution of the protein in the micellar detergent phase. Even though increasing [Det]$_w$ moves the protein population towards a more dissociated state (Figure 1), the underlying cause is not due to an increase of the detergent concentration in the micellar phase, [Det]$_{mic}$. Rather, it is the volume of the micellar phase that changes as a function of [Det]$_w$. It is this change in volume that influences the oligomeric protein distribution: it is a simple dilution effect.

The advantage of quantifying transmembrane protein interaction energetics is that this simple dilution effect is easily understood and can be used to adjust experimental conditions to allow precise tuning of the equilibrium population. Figure 5(a) shows the true equilibrium distribution for the SNGp99 transmembrane helix dimer in micellar C_8E_5 on the mole fraction scale. Figure 5(b) shows the projection of this information onto the bulk aqueous scale for two different aqueous detergent concentrations. In Figure 5(b), it can be seen that, at a bulk protein concentration of 1 μM, the equilibrium population contains ~80% dimer in a solution of 11 mM [C_8E_5]$_w$. Upon adjustment of the solution conditions to contain 146 mM [C_8E_5]$_w$, the equilibrium distribution shifts to 40% dimeric protein. The ability to predict these shifts in the oligomeric distribution will allow the equilibrium ensemble to be optimized in order to favor experimental conditions that may be desirable for addressing specific scientific questions. An attractive strategy may be the ability to compare structural properties between the monomeric and oligomeric forms of transmembrane helix–helix oligomers by judiciously manipulating the bulk aqueous concentrations of protein and micellar detergent.

Conclusions

The assumptions behind the protein–micellar detergent mole fraction standard state free energy calculation have been explored. The theoretical expressions incorporate the

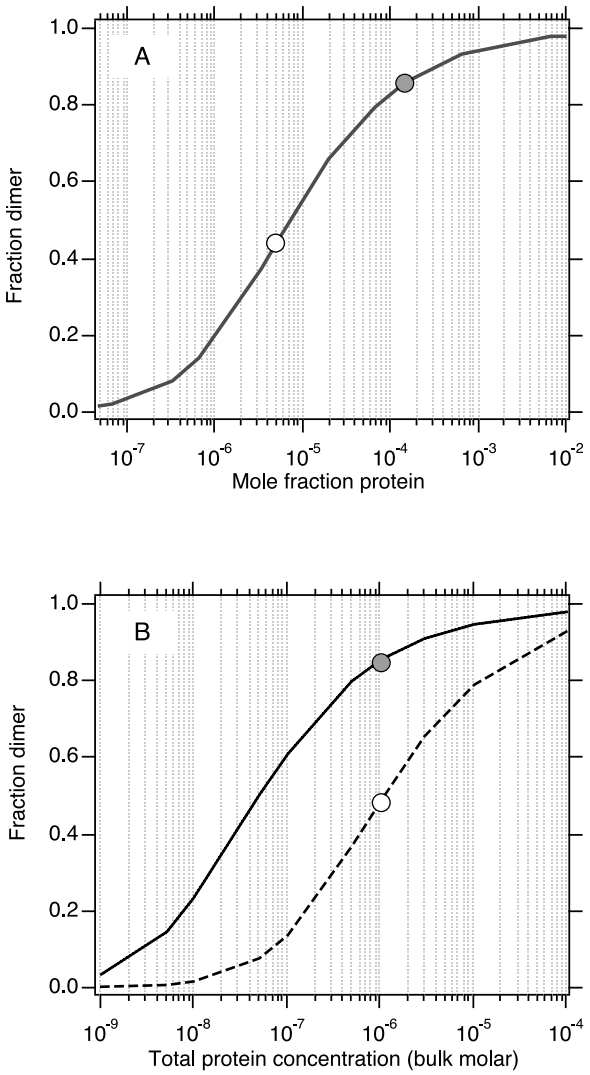

Figure 5. Modulation of protein equilibrium ensemble by dilution. (a) A graphical representation of the dilution effect: the distribution of dimeric SNGpA99 in micellar C_8E_5 at 25 °C is given on the mole fraction scale. (b) The distribution of dimeric SNGpA99 in bulk molar units is shown for two aqueous detergent concentrations (also in bulk molar units: 11 mM $[C_8E_5]_w$, continuous line, and 146 mM $[C_8E_5]_w$, broken line). The open and shaded circles show how equivalent oligomeric population distributions are expressed on both scales.

assumption that the change in the chemical potential upon transmembrane protein dimerization can be assigned to changes in the protein–protein association reaction in micellar detergent phase. Thus the change in the chemical potential of the water phase is assigned a value of zero. In the case of ideal-dilute solution behavior for the two component protein–micellar detergent phase, the $\ln K_{A,app}$ experimentally obtained bulk molar units should vary linearly as a function of ln[micellar Det]$_w$ with a slope of negative unity. When this is found, equation (1) can be used in a straightforward manner to calculate the standard state free energy change of association. If a concentration-dependent potential energy term is significant, a further modulation of the reaction energetics will be found, and the standard state free energy change may be calculated incorrectly unless this is taken into account.

The clear ability to calculate a standard state free energy change should facilitate further partitioning of the energetics of transmembrane protein–protein associations in hydrophobic environments. Of particular interest will be an examination of the effect of the protein sequence and the hydrophobic environment on the value of the standard state free energy change. Emerging from such studies should be an improved understanding of the physical and chemical basis for transmembrane helix–helix interactions in hydrophobic solvents.

Materials and Methods

Sample preparation and analytical ultracentrifugation

SNGpA99 is a chimeric protein comprised of staphylococcal nuclease followed by the transmembrane domain of human glycophorin A (GpA). This fusion protein has been used extensively by Engelman and co-workers to explore the sequence-dependence of GpA transmembrane dimerization.[4–6,10] The fusion protein has been described in detail and was purified using published protocols.[6] Immediately before sedimentation equilibrium analysis, samples were exchanged by ion-exchange chromatography into buffer containing C_8E_5 as described,[6] except that the final detergent concentration for each experiment is indicated.

Sedimentation equilibrium experiments were performed at 25 °C using a Beckman XL-A analytical ultracentrifuge as described.[6,31,32] The samples were centrifuged for lengths of time sufficient to achieve equilibrium. Data obtained from absorbance at 230 nm were analyzed by non-linear least-squares curve fitting of radial concentration profiles using the Windows version of NONLIN[33] using the equations describing the reversible association in sedimentation equilibrium. The seven concentrations of detergent employed were 11 mM, 15 mM, 23 mM, 33 mM, 50 mM, 96 mM and 146 mM C_8E_5. The concentration of detergent in micelles was calculated by subtracting the critical micelle concentration (4.3 mM) from each of the total detergent numbers above. For each global fit (at each concentration of detergent), nine equilibrium data sets were collected. These consisted of three different initial protein concentrations analyzed at three significantly different speeds (20,000, 24,500, and 30,000) i.e. such that the speed factor ratios were minimally 1.0, 1.5 and 2.25. The monomeric molecular masses and partial specific volumes were calculated using the program SEDINTERP,[34] and these parameters were held constant in fitting the absorbance *versus* radius profiles.

Acknowledgements

This work was supported by a grant from the National Institutes of Health, GM57534. The author

thanks members of the Fleming laboratory for helpful conversations.

References

1. Liu, J. & Rost, B. (2001). Comparing function and structure between entire proteomes. *Protein Sci.* **10**, 1970–1979.
2. White, S. H. & Wimley, W. C. (1999). Membrane protein folding and stability: physical principles. *Annu. Rev. Biophys. Biomol. Struct.* **28**, 319–365.
3. Popot, J.-L. & Engelman, D. M. (1990). Membrane protein folding and oligomerization: the two-stage model. *Biochemistry*, **29**, 4032–4037.
4. Lemmon, M. A., Flanagan, J. M., Hunt, J. F., Adair, B. D., Bormann, B. J., Dempsey, C. E. *et al.* (1992). Dimerization is driven by specific interactions between transmembrane α-helices. *J. Biol. Chem.* **267**, 7683–7689.
5. Lemmon, M. A., Flanagan, J. M., Treutlein, H. R., Zhang, J. & Engelman, D. M. (1992). Sequence specificity in the dimerization of transmembrane α-helices. *Biochemistry*, **31**, 12719–12725.
6. Fleming, K. G., Ackerman, A. L. & Engelman, D. M. (1997). The effect of point mutations on the free energy of transmembrane α-helix dimerization. *J. Mol. Biol.* **272**, 266–275.
7. Arkin, I. T., Adams, P. D., MacKenzie, K. R., Lemmon, M. A., Brunger, A. T. & Engelman, D. M. (1994). Structural organization of the pentameric transmembrane α-helices of phospholamban, a cardiac ion channel. *EMBO J.* **13**, 4757–4764.
8. Fisher, L. E., Engelman, D. & Sturgis, J. (1999). Detergents modulate dimerization, but not helicity, of the glycophorin A transmembrane domain. *J. Mol. Biol.* **293**, 639–651.
9. Gratkowski, H., Lear, J. D. & DeGrado, W. F. (2001). Polar side chains drive the association of model transmembrane peptides. *Proc. Natl Acad. Sci. USA*, **98**, 880–885.
10. Fleming, K. & Engelman, D. (2001). Specificity in transmembrane helix–helix interactions defines a hierarchy of stability for sequence variants. *Proc. Natl Acad. Sci. USA*, **98**, 14340–14344.
11. Zhou, F. X., Cocco, M. J., Russ, W. P., Brunger, A. T. & Engelman, D. M. (2000). Interhelical hydrogen bonding drives strong interactions in membrane proteins. *Nature Struct. Biol.* **7**, 154–160.
12. Zhou, F. X., Merianos, H. J., Brunger, A. T. & Engelman, D. M. (2001). Polar residues drive association of polyleucine transmembrane helices. *Proc. Natl Acad. Sci. USA*, **98**, 2250–2255.
13. Bu, Z. & Engelman, D. M. (1999). A method for determining transmembrane helix association and orientation in detergent micelles using small angle X-ray scattering. *Biophys. J.* **77**, 1064–1073.
14. Adair, B. D. & Engelman, D. M. (1994). Glycophorin A helical transmembrane domains dimerize in phospholipid bilayers: a resonance energy transfer study. *Biochemistry*, **33**, 5539–5544.
15. Kochendoerfer, G. G., Salom, D., Lear, J. D., Wilk-Orescan, R., Kent, S. B. & DeGrado, W. F. (1999). Total chemical synthesis of the integral membrane protein influenza A virus M2: role of its C-terminal domain in tetramer assembly. *Biochemistry*, **38**, 11905–11913.
16. Choma, C., Gratkowski, H., Lear, J. D. & DeGrado, W. F. (2000). Asparagine-mediated self-association of a model transmembrane helix. *Nature Struct. Biol.* **7**, 161–166.
17. Langosch, D., Brosig, B., Kolmar, H. & Fritz, H.-J. (1996). Dimerisation of the glycophorin A transmembrane segment in membranes probed with the ToxR transcription activator. *J. Mol. Biol.* **263**, 525–530.
18. Laage, R. & Langosch, D. (1997). Dimerization of the synaptic vesicle protein synaptobrevin (vesicle-associated membrane protein) II depends on specific residues within the transmembrane segment. *Eur. J. Biochem.* **249**, 540–546.
19. Brosig, B. & Langosch, D. (1998). The dimerization of the glycophorin A transmembrane segment in membranes: importance of glycine residues. *Protein Sci.* **7**, 1052–1056.
20. Russ, W. P. & Engelman, D. M. (1999). TOXCAT: a measure of transmembrane helix association in a biological membrane. *Proc. Natl Acad. Sci. USA*, **96**, 863–868.
21. Laage, R., Rohde, J., Brosig, B. & Langosch, D. (2000). A conserved membrane-spanning amino acid motif drives homomeric and supports heteromeric assembly of presynaptic SNARE proteins. *J. Biol. Chem.* **275**, 17481–17487.
22. Russ, W. P. & Engelman, D. M. (2000). The GxxxG motif: a framework for transmembrane helix–helix association. *J. Mol. Biol.* **296**, 911–919.
23. Gurezka, R. & Langosch, D. (2001). *In vitro* selection of membrane-spanning leucine zipper protein–protein interaction motifs using POSSYCCAT. *J. Biol. Chem.* **276**, 45580–45587.
24. Senes, A., Gerstein, M. & Engelman, D. M. (2000). Statistical analysis of amino acid patterns in transmembrane helices: the GxxxG motif occurs frequently and in association with beta-branched residues at neighboring positions. *J. Mol. Biol.* **296**, 921–936.
25. Salom, D., Hill, B. R., Lear, J. D. & DeGrado, W. F. (2000). pH-Dependent tetramerization and amantadine binding of the transmembrane helix of M2 from the influenza A virus. *Biochemistry*, **39**, 14160–14170.
26. Li, R., Babu, C. R., Lear, J. D., Wand, A. J., Bennett, J. S. & DeGrado, W. F. (2001). Oligomerization of the integrin alphaIIbbeta3: roles of the transmembrane and cytoplasmic domains. *Proc. Natl Acad. Sci. USA*, **98**, 12462–12467.
27. Tanford, C. (1991). Mixed micelles. In *Hydrophobic Effect: Formation of Micelles and Biological Membranes*, Krieger Publishing Company, chapt. 9. pp. 90–95, Malabar, FL.
28. Atkins, P. (1999). *Physical Chemistry*, W.H. Freeman and Company, New York.
29. Gurney, R. W. (1953). *Ionic Processes in Solution*, Dover Publications, Inc, New York.
30. Tinoco, I. J., Sauer, K. & Wang, J. C. (1978). *Physical Chemistry: Principles and Applications in Biological Sciences*, Prentice-Hall Inc, Upper Saddle River.
31. Fleming, K. G. (1998). Measuring transmembrane α-helix energies using analytical ultracentrifugation. In *Chemtracts: Biological Applications of the Analytical Ultracentrifuge* (Hanson, J. C., ed.), vol. 11, pp. 985–990, Springer, New York.
32. Fleming, K. G. (2000). Probing the stability of helical membrane proteins. *Methods Enzymol.* **323**, 63–77.
33. Johnson, M. L., Correia, J. J., Yphantis, D. A. & Halvorson, H. R. (1981). Analysis of data from the analytical ultracentrifuge by nonlinear least-squares techniques. *Biophys. J.* **36**, 575–588.

34. Laue, T. M., Shah, B., Ridgeway, T. M. & Pelletier, S. L. (1992). Computer-aided interpretation of analytical sedimentation data for proteins. In *Analytical Ultracentrifugation in Biochemistry and Polymer Science* (Harding, S. E., Rowe, A. J. & Horton, J. C., eds), pp. 90–125, Royal Society of Chemistry, Cambridge.

Edited by G. von Heijne

(Received 17 June 2002; received in revised form 20 August 2002; accepted 25 August 2002)