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Protein Stabilization by Naturally Occurring Osmolytes

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1. Introduction

Natural selection is believed to be an unforgiving and relentless force in the evolution of life on earth. An organism that cannot adapt to a changing environment or an environment hostile to cell functions is at risk as a species. So it is important to understand the mechanisms used by plants, animals, and microorganisms in adapting to environments in the biosphere that would ordinarily denature proteins or otherwise cause disruption of life-giving cellular processes. These hostile environments involve such stresses as extremes of temperature, cellular dehydration, desiccation, high extracellular salt environments, and even the presence of denaturing concentrations of urea inside cells ***(1)***. It has been recognized for some time that many plants, animals, and microorganisms that have adapted to environmental extremes also accumulate significant intracellular concentrations of small organic molecules ***(1–4)***. From these (and other) observations comes the hypothesis that these small organic molecules, called osmolytes, have the ability to protect the cellular components against denaturing environmental stresses ***(1–5)***. In this chapter, we seek to understand the molecular-

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level phenomena involving proteins and the naturally occurring osmolytes that result in the stabilization of proteins against denaturation stresses.

The disaccharide trehalose is the principal osmolyte selected to protect the resurrection plant against desiccation, and it is the osmolyte selected by nature to protect tardigrades (primitive arthropods) against the deleterious effects of desiccation ***(6,7)***. This is only one example in which organisms from different kingdoms have been subjected to the same selective pressure (desiccation) and have settled on the same osmolyte for protection against that stress. Such cases have been cited as examples of convergent evolution ***(1)***. If convergent evolution indeed selects for particular organic compounds as cell-component protectants, then the property or properties of the osmolyte that bestow the protection must be highly valued evolutionarily. Identification of an evolutionarily selected property or properties of osmolytes that protect proteins against denaturing stresses should provide valuable insight into the fundamental problem of protein stabilization, a problem that plagues the pharmaceutical industry, biotechnology, and everyday research.

Satisfying biological needs and biological constraints are a necessity in adaptation, and it is most enlightening to approach the issue of protein stabilization in that context. In the natural selection of a mechanism that will enable an organism to adapt to denaturing stresses, two conditions must be met: (1) the mechanism must provide for the stabilization of cellular proteins and other cell components against the denaturing environmental stress; and (2) the mechanism must provide the stabilization without significantly affecting the functional activity of the proteins and other cellular components ***(1,8,9)***. This places stringent constraints on the types of mechanisms appropriated through natural selection. The condition of not affecting the functional activity of proteins and other cellular components is just as important as providing for stabilization, for there is no selective advantage for a mechanism that solves the stabilization problem while creating problems with cellular function. Our goal in this chapter is to investigate how physical and/or chemical

properties of osmolytes may be responsible for the stabilization of proteins, and to relate these properties to the biology of adaptation.

2. Naturally Occurring (Protecting and Nonprotecting) Osmolytes

Intracellular accumulation of organic solutes creates an osmotic pressure that can affect cell volume, and this osmotic effect is the reason these solutes are often referred to as organic osmolytes ***(10)***. Small organic osmolytes were selected by nature to protect the cell and cellular components against particular denaturing stresses; however, not all osmolytes protect cell components. An important nonprotecting osmolyte is urea, which accumulates in mammalian renal medulla cells and also in the cells of sharks, rays, and the coelacanth ***(1)***. The intracellular proteins in urea-enriched cells are just as susceptible to the strong denaturing properties of urea as the proteins in cells that do not accumulate urea. What permits urea-rich cells to thrive is the intracellular presence of methylamine osmolytes—compounds that have the ability to offset (i.e., protect against) the deleterious effects of urea ***(8,11,12)***. Urea is an osmolyte in a class by itself—it is a nonprotecting osmolyte.

In contrast to urea, the array of naturally occurring organic osmolytes that are classified as protecting osmolytes fall into three general chemical classes: (1) the polyols, which include glycerol, sucrose, trehalose, and certain other sugars; (2) certain amino acids, including proline and glycine; and (3) particular methylamines, such as sarcosine, trimethylamine N-oxide (TMAO), and betaine. A more extensive listing of protecting osmolytes can be found in ***(1)***. Assuming that environmental stresses are the sources of selective pressure for the accumulation of organic osmolytes in an organism, particular classes of osmolytes appear to have been selected for their ability to oppose particular stress conditions. For example, polyols appear to be particularly good at protecting organisms against stresses such as temperature extremes and dehydration or desiccation, and amino acids appear to have been selected to protect cells

against extracellular environments that are high in salt concentration ***(1)***. By contrast, the methylamine osmolytes seem to have the ability to protect the cellular components of urea-rich cells against the deleterious effects of urea ***(1)***.

A general conclusion that might be drawn from these observations is that protecting osmolytes are stress-specific, and one should not expect an osmolyte that is good at protecting against one denaturing stress to be effective in protecting against a different denaturing stress. Yet it would be wrong to conclude that a different basic mechanism for protection is operative with each protecting osmolyte or osmolyte class. There is considerable similarity in the mechanism by which the organic osmolytes protect against denaturing stresses—a commonality that reveals itself in the property of preferential exclusion ***(13–24)***.

3. Essential Physico-Chemical Observations

3.1. Preferential Exclusion: A Common Feature of Protecting Osmolytes

Sucrose has long been used in biochemistry to stabilize proteins, and in an effort to understand this process, Lee and Timasheff ***(25)*** evaluated the binding of sucrose to proteins by equilibrium dialysis. Favorable binding of a ligand to protein results in the ligand concentration in the protein-containing compartment that is the sum of the concentrations of unbound ligand and ligand bound to protein, while in the compartment lacking protein only unbound ligand concentration is possible. At equilibrium, the chemical potentials of unbound ligand in both compartments are identical. Thus, favorable ligand binding results in a greater concentration of ligand in the protein-containing compartment than in the compartment lacking protein.

In their equilibrium dialysis experiment, Lee and Timasheff found that as a ligand, sucrose is observed to have a greater concentration in the compartment lacking protein than it has in the compartment containing protein—i.e., the results are the exact opposite

of those expected for favorable ligand binding to protein ***(25)***. This result unequivocally shows that sucrose does not bind to proteins, and it is preferentially excluded from the vicinity of the protein. This preferential exclusion phenomenon has been found for the naturally occurring protecting osmolytes and is a common defining feature of these solutes.

Obviously, preferential exclusion of a ligand from the immediate vicinity of a protein implies that the concentration of ligand in the immediate volume element surrounding the protein is lower than its concentration in the bulk phase. Clearly, if ligand concentration is lower in this volume element than in the bulk phase, then water concentration in this volume element necessarily must be higher than it is in the bulk phase. Thus, preferential exclusion of ligand in the volume element is equivalent to saying the protein is preferentially hydrated. Thus, the terms *preferential exclusion* of ligand and *preferential hydration* of the protein refer to the same phenomenon, and are often used interchangeably.

3.2. Gibbs Energy Implications of Preferential Exclusion

By definition, favorable ligand binding to a protein always results in a thermodynamic decrease in the Gibbs energy for the process—i.e., the standard chemical potential for the interacting protein-ligand complex is lower than the sum of the individual standard chemical potentials of free ligand and free protein ($\Delta G°$ is negative). By contrast, preferential exclusion causes an increase in $\Delta G°$, and the standard chemical potential of the sucrose-protein system is greater than the sum of the standard chemical potentials of free ligand and free protein ***(26,27)***. Using transfer Gibbs energy measurements, we have shown that the thermodynamic effect of stabilizing osmolytes on the equilibrium between native and unfolded protein gives a unique Gibbs energy diagram that illustrates how protein stabilization arises from preferential exclusion ***(27–29)***. Arakawa and Timasheff originally presented the basic features of the Gibbs energy diagram, reproduced here in **Fig. 1**, and based on preferential exclusion.

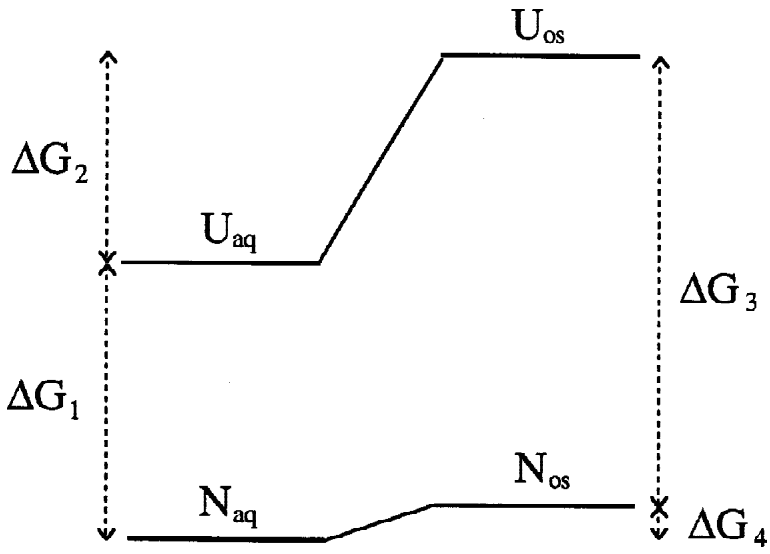

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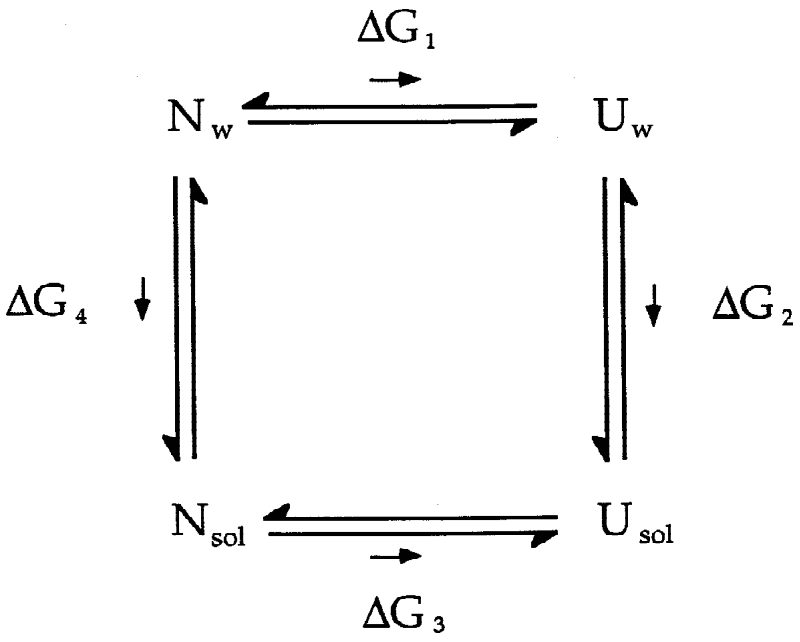
Fig. 1. (Reprinted with permission from **ref. *28***.)

measurements ***(13)***. In fact, Timasheff and colleagues ***(30)*** have clearly shown the thermodynamic relationship between transfer Gibbs energy and the preferential interaction parameters measured and used in their approach. **Figure 1** identifies four processes represented by the unfolding reaction of native protein in aqueous solution to unfolded protein in aqueous solution ($\Delta G°_1$); the transfer of unfolded protein in aqueous solution to unfolded protein in (for example) 1 *M* protecting osmolyte solution ($\Delta G°_2$); the unfolding reaction of native protein in 1 *M* osmolyte solution to unfolded protein in 1 *M* protecting osmolyte solution ($\Delta G°_3$); and the transfer of native protein in aqueous solution to native protein in 1 *M* protecting osmolyte solution (ΔG_4). These events comprise the thermodynamic cycle presented in **Scheme 1**.

Several conclusions can be drawn from **Fig. 1**. First, the stabilization by protecting osmolyte does not arise from osmolyte stabilization of the native state. In fact, the Gibbs energy diagram shows that transferring native protein from water to 1 *M* protecting osmolyte solution increases the Gibbs energy, meaning that the ($\Delta G°_4$) transfer has a positive sign and transfer is therefore desta-



Scheme 1

bilizing ***(27)***. Second, the Gibbs energy of transfer the unfolded state of the protein from aqueous solution to 1 *M* osmolyte ($\Delta G°_2$) is increased much more than ($\Delta G°_4$), suggesting that exposure of the protein fabric to osmolyte solution is a solvophobic process. Third, protein unfolding in 1 *M* osmolyte solution is more unfavorable than unfolding in aqueous solutions ($\Delta G°_3$ is more positive than $\Delta G°_1$). This is a direct consequence of the relatively large unfavorable transfer of unfolded protein from water to 1 *M* osmolyte solution ***(13,26–29)***. Thus, osmolyte stabilization of proteins arises principally from the destabilization of the unfolded state of the protein in the presence of osmolyte. The importance of this mechanism is that osmolytes act primarily on the denatured state in bringing about stabilization, while leaving the native state relatively unaffected and functional (through preferential exclusion of osmolyte). This mechanism provides the means for satisfying the two conditions for natural selection—i.e., stabilizing proteins against the denaturing stress—while not interfering with the functional activity of macromolecules.

4. Transfer Gibbs Energy

From **Fig. 1**, it is clear that the (unfavorable) solvophobic effect of transferring unfolded protein from water to 1 *M* osmolyte solution is critical to understanding the chemical origin of the stabilization of proteins by protecting osmolytes. Thus, identification of the part or parts of the protein responsible for the solvophobic effect is extremely helpful in identifying the nature of the force or forces responsible. The method best suited for identifying the relative favorability or unfavorability of transferring protein functional groups from water to osmolyte solutions involves the measurements of transfer Gibbs energies (Δg°_{tr}). Δg°_{tr} gives a measure of the preference of that functional group for interaction with the cosolvent species relative to its preference for interaction with water ***(31,32)***. Transfer Gibbs energy measurements have a long and storied history in providing an understanding of how strong denaturants such as urea and guanidinium chloride work their magic, and in identifying and characterizing such important fundamental thermodynamic forces as the hydrophobic effect ***(31–33)***. For the purpose of understanding how protecting osmolytes work, transfer Gibbs energy changes are the measurements of choice.

4.1. Theory Behind Transfer Gibbs Energy Measurements

First, a word about terminology. Let's say we want to determine whether some particular functional group on the protein—for example, the peptide backbone—contributes to the solvophobic effect observed on transfer of an unfolded protein from water to 1 *M* sucrose. We then evaluate the Gibbs energy of transfer of the peptide backbone from water to 1 *M* sucrose. The sign of the Gibbs energy change on transfer (Δg°_{tr}) of the peptide backbone from water to 1 *M* sarcosine tells us with which of the two solvent components the backbone would rather be in contact. And the magnitude of the Δg°_{tr} tells us by how much (in energy terms) the backbone prefers the favored solvent. It is important to note that water is always taken to be the reference-state solvent, and an unfa-

vorable Δg°_{tr} means that when given a choice of the two solvents, the peptide backbone prefers to interact with water more than it does with 1 *M* sucrose. Later in this chapter, we will find that the Δg°_{tr} of the peptide backbone from water to protecting osmolyte solutions is indeed unfavorable, and from the positive sign of Δg°_{tr}, it is common to say that the osmolyte interacts unfavorably with the peptide backbone. This statement means that relative to the interaction of water with the peptide backbone, osmolyte solution interacts unfavorably with the backbone. The transfer of peptide backbone from water to protecting osmolyte solution is a solvophobic effect—it is solvophobic with respect to water as the reference solvent ***(28)***.

The application of transfer Gibbs energy measurements to proteins was advanced in development in the 1930s ***(34,35)***. There are many variations of the method, and the one we have used is based on the solubility measurements of amino acids in water and in the specified osmolyte solution. **Fig. 2** shows solubility plots of alanine in water and alanine in 1 *M* TMAO ***(27)***. The limits of solubility of alanine in water and in 1 *M* TMAO are represented by the alanine concentrations at the intersections of the two lines in each solubility plot. At these solubility limits, the chemical potential of the amino acid in the crystal is equal to the chemical potential of the amino acid in the solvent. Thus, for the two experiments we can write:

$$\mu(\text{AA crystal}) = \mu(\text{AA in water}) \quad (1)$$

$$\mu(\text{AA crystal}) = \mu(\text{AA in 1 } M \text{ TAMO}) \quad (2)$$

Because the chemical potential of the amino acid in the crystal is the same in both experiments we can write:

$$\mu(\text{AA in water}) = \mu(\text{AA in 1 } M \text{ TAMO}) \quad (3)$$

And this equation can be expanded in the usual manner to give:
$\mu^\circ(\text{AA in water}) + RT \ln a(\text{AA in water}) =$

$$\mu^\circ(\text{AA in 1 } M \text{ TAMO}) + R\,T \ln a(\text{AA in 1 } M \text{ TAMO}) \quad (4)$$

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where a(AA in water) and a(AA in 1 *M* TMAO) represent the activities of the amino acid in water and 1 *M* TMAO respectively.

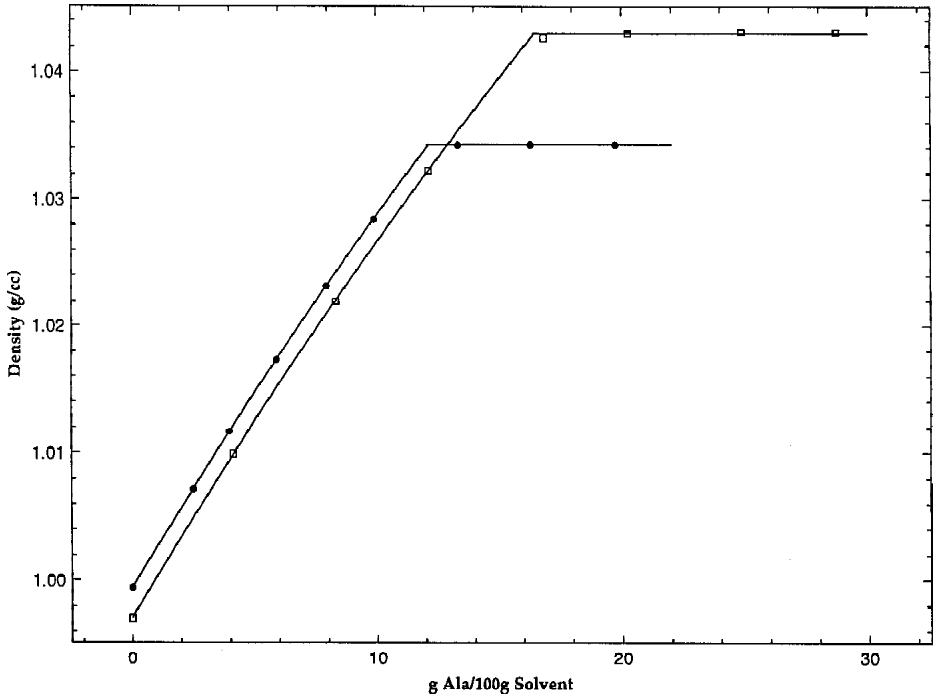

Fig. 2. Density of supernatant solutions vs composition of alanine in water and 1 *M* TMAO solutions. Solubility limits of alanine in water (open squares) and in 1 *M* TMAO (filled circles) are determined at the intersection points of the solid lines.

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Collecting terms in the above equation gives:

$$\Delta\mu^\circ = R\ T\ \ln [a(\text{AA in 1 } M \text{ TAMO})/a(\text{AA in water})]$$
$$\text{where } \Delta\mu^\circ = \mu^\circ(\text{AA in 1 } M \text{ TAMO}) - \mu^\circ(\text{AA in water}) \quad (5)$$

It is very difficult to evaluate the activities of the individual components of a three-component mixture, so activities are commonly taken as molar concentrations and the equation becomes:

$$\Delta G^\circ_{tr} = \Delta m^\circ = R\ T\ \ln [C(\text{AA in 1 M TMAO})/C(\text{AA in water})]$$

where C(AA in 1 *M* TAMO) is the molar concentration of the amino acid at the solubility limit in 1 *M* TMAO, C(AA in water) is the amino-acid (molar) concentration at the solubility limit in water,

and $\Delta G°_{tr}$ is the standard transfer Gibbs energy of the amino acid from water to 1 *M* TMAO ***(29,36,37)***.

4.2. Methods

Transfer Gibbs energy values for 20 common amino acids can be evaluated from solubility measurements such as those shown in **Fig. 2**. From the tabulated values of $\Delta G°_{tr}$, the Gibbs energy of transfer of side chains ($\Delta g°_{tr}$) may be estimated by subtracting $\Delta G°_{tr}$ of glycine from all other amino acids ***(32,38)***. To determine $\Delta g°_{tr}$ for the peptide backbone, the solubilities of the cyclic glycylglycine model compound, diketopiperazine (DKP) can be evaluated in water and in a solution of fixed osmolyte concentration. The resulting transfer Gibbs energy of DKP from water to fixed osmolyte concentration is divided by two in order to express the transfer Gibbs energy on a per mol peptide backbone basis ***(27)***. The tabulated $\Delta g°_{tr}$ values for the side chains and the peptide backbone serve as the database for evaluating the effects of osmolyte on protein denaturation.

5. The Transfer Model and Protein Folding

5.1. What is Being Modeled?

Scheme 1 succinctly outlines the relationship between transfer and protein stability, with $\Delta G°_4$ and $\Delta G°_2$ representing the Gibbs energies of transfer of native state and denatured state from water to cosolvent solution, respectively. These transfer processes are related to the unfolding Gibbs energy changes in water ($\Delta G°_1$) and in osmolyte solution ($\Delta G°_3$) by **Eq. 6**:

$$\Delta G°_2 - \Delta G°_4 \rightleftarrows \Delta G°_3 - \Delta G°_1 \qquad (6)$$

which shows that by determining the transfer of native state ($\Delta G°_4$) or denatured state ($\Delta G°_2$) from water to osmolyte solution, one can obtain a measure of the increase in the protein's stability in osmolyte over its stability in water ($\Delta G°_3 - \Delta G°_1$).

By means of preferential interaction measurements, Lin and Timasheff ***(30)*** experimentally determined $\Delta G°_2$ and $\Delta G°_4$ for ribo-

nuclease T1. But another way of obtaining $\Delta G°_2$ and $\Delta G°_4$ is to estimate these quantities by use of models of the denatured and native protein. The latter approach, called the Transfer Model, was used by Nozaki and Tanford to obtain insight into how strong cosolvent denaturants such as urea and guanidine hydrochloride destabilize proteins ***(32,38–40)***. The advantage of using the Transfer Model is that it identifies and quantifies which functional groups on the native and denatured states of the protein being transferred from water to cosolvent solution are principally responsible for the differences in protein stability in the cosolvent solution relative to the protein's stability in water. Much of what we know about the efficacies of urea and guanidine hydrochloride and their interactions with protein functional groups originates from Nozaki and Tanford's pioneering use of the Transfer Model to understand urea and guanidine hydrochloride denaturation ***(38)***. In this chapter, we have adopted the Transfer Model for the purpose of understanding the energetics of interactions between osmolytes and groups on the protein that protect proteins against denaturation.

Operationally, the Transfer Model consists of identifying the solvent-accessible functional groups on the native and denatured forms of the protein; quantifying the fraction of solvent-exposed surface area; multiplying the fractions of solvent-exposed functional groups by their Gibbs energies of transfer; and summing all of the transfer Gibbs energy contributions to obtain estimates of the transfer Gibbs energy of the native ($\Delta G°_4$) or denatured species ($\Delta G°_2$) from water to the specific cosolvent solution. The last step requires appropriate models for the denatured and native protein species, and appropriate models for determining the Gibbs energy of transfer of side chain and backbone functional groups. Such models are described in **Subheading 5.2.**

5.2. Assumptions of the Transfer Model

Amino-acid transfer Gibbs energy measurements have had a long and important history, and they have played a key role in understanding the effect of denaturants and organic solvents on protein

stability ***(31–35,38–44)***. The basic idea for use of these quantities is that the sum of the Gibbs energy of transfer of the solvent-exposed component parts of a protein from water to cosolvent equals the Gibbs energy of transfer of the whole protein. This view is based on the assumption that the contributions of component parts of the protein are additive, and this assumption is one of the weaknesses of the model. Several investigators have struggled with the question of additivity with much, but not all, of the data in support of the concept of additivity of side-chain contributions ***(32,38,40,45–50)***. Similarly, additivity of the peptide backbone unit appears to depend on chain length, and while some have presented data indicating the transfer Gibbs energy of peptide backbone unit is additive, others suggest nonadditivity ***(32,38,40,46,47)***. Because of the long history of the issue of additivity, it is doubtful that a convincing case, could settle the issue without extensive studies using a wide variety of compounds and solvent systems. Our view is that additivity for side chain and backbone units occurs to a significant extent—or the ability to obtain reasonable unfolding Gibbs energy changes based upon side-chain and backbone-transfer free-energy data would not be as successful as it continues to be ***(43,44)***. It should be noted that additivity of side-chain transfer free-energy data of Nozaki and Tanford ***(51)*** is incorporated as an essential part of theories that have been successful in modeling the thermodynamics of protein folding.

In addition to additivity, several other assumptions and approximations affect the magnitude of Gibbs energies obtained from application of the Transfer Model. As previously mentioned, activity coefficients for three-component systems are so difficult to evaluate that we have little choice but to use concentrations rather than activities to determine transfer Gibbs energies ***(36,37,52)***. Also, Nozaki and Tanford have discussed the issue of what constitutes a good model of the peptide backbone, and several different models have been used to evaluate the transfer Gibbs energy of the peptide backbone ***(33,39,40,42)***. The model we use—diketopiperazine (DKP, alternatively known as cyclic glycylglycine)—is found to give transfer Gibbs energies within 10–15% of those reported for the preferred model used by Nozaki and Tanford ***(27)***. Thus, the

transfer Gibbs energy for the peptide backbone may also be a potential source of some error.

Despite the various assumptions and approximations used in the Transfer Model, the Gibbs energy of transfer of native and denatured RNase T1 from water to 1 *M* TMAO obtained from the Transfer Model ($\Delta G°_4$ = 1.7 and $\Delta G°_2$ = 5.9 kcal/mol) agree favorably with the respective values of 1.2 and 5.4 kcal/mol determined experimentally by Lin and Timasheff ***(29,30)***. The agreement provides evidence that the approximations and assumptions are not debilitating to the model, and it also provides a degree of confidence that the major factors responsible for protein stabilization by osmolytes can be identified from transfer data.

Despite the assumptions and approximations of the Transfer Model, it has contributed prominently to the development of the concept of hydrophobic interactions, in understanding denaturant-induced denaturation, and in issues involving protein stabilization ***(27–29,31, 41,49,50,53)***. The strength of the Transfer Model is that from its application, one can identify the major solvent-protein fabric interactions responsible for protein stability. It is important to have a clear understanding of the limitations of the Transfer Model inherent in the assumptions and approximations, and to avoid making too much of it quantitatively. With these conditions, use of the Model provides insight into a number of fundamental principles of protein folding.

5.3. Implementation of the Transfer Model

With tabulated values of side-chain and backbone-unit transfer Gibbs energies from water to osmolyte solutions of fixed concentration, one needs only the crystal coordinates of a protein of interest to transfer the native state from water to cosolvent. Operationally, $\Delta G°_4$ and $\Delta G°_2$ are evaluated according to **Eq. 7**, where $\Delta G°_i$ represents the standard-state transfer Gibbs energy of the peptide backbone U and each of the various amino-acid side chains, n_i is the number of residues of amino acid "i" in the protein, $\Delta G°_i$ is the transfer Gibbs energy of the side chain of residue of type "i", and α_i is the mean fractional accessibility of the n_i amino-acid

side chains or peptide backbone units in the (native or unfolded) protein species of interest ***(27)***.

$$\Delta G^\circ_{2 \text{ or } 4} = \Sigma\, n_i \alpha_i \Delta g^\circ_i \qquad (7)$$

To illustrate a calculation of minimal sophistication, let's say we want to evaluate α for the side chain of alanine in the native state of a protein which has a total of 11 alanine residues. Of these, only five have alanine side chains accessible to solvent, each with varying degrees of solvent exposure. The term $\alpha_{(\text{ala side chain})}$ for the alanine side chains is obtained by summing the solvent accessible surface areas contributed by the five alanine side chains that are partially accessible to solvent. This sum is then divided by a standard-state accessible surface area for the alanine side chain derived from the extended tripeptide gly-ala-gly, with dihedral angles $\Phi = -140°$, $\Psi = 135°$, $X = -120°$ ***(54,55)***.†

The quantity resulting from this interim numerical evaluation ($\alpha^*_{(\text{ala side chain})}$) represents the number of alanine side chains fully accessible to solvent. Dividing this number by 11 gives the mean (fractional) exposure of alanine side chains accessible to solvent in the native state of the protein, $\alpha_{(\text{ala side chain})}$. Such calculations are performed for the side chains of all 20 amino acids in the protein plus the peptide backbone, and the α_i values are used in **Eq. 7** to give ΔG°_4 , the transfer Gibbs energy of the native state of the protein from water to the osmolyte.

Although a variety of programs exist for evaluating the accessible surface area (α_i values), we have used the static accessible surface area algorithm of Lee and Richards as modified by Lesser & Rose, taking 1.4 Å*s* as the probe size of the solvent ***(55,56)***. While the crystallographic coordinates of the protein provides a model of the native state of the protein, the lack of defined structure for the denatured state makes it necessary to model the denatured ensemble. Creamer et al. ***(57)*** examined the complexities of the denatured ensemble, and have suggested two limiting models that bracket the expected behavior of an unfolded chain. One limiting model of the denatured state, which sets the upper boundary of solvent accessi-

bility, involves a hard sphere model (with attending excluded volume effects) that behaves as a homopolymer in a good solvent. That is, the model representing the upper boundary is highly solvent-accessible. The limiting model that sets the lower boundary of solvent accessibility is generated from excised fragments of folded proteins that retain intramolecular interactions. This model is representative of a compact denatured ensemble. **Equations 8** and **9** provide the means to estimate the Gibbs energy changes for transfer of

$$\Delta G^\circ_{tr\,lb} = \Sigma \Delta g^\circ_i * n_i * \alpha_i * (\text{lower ASA/stand ASA}) \quad (8)$$

$$\Delta G^\circ_{tr\,ub} = \Sigma \Delta g^\circ_i * n_i * \alpha_i * (\text{upper ASA/stand ASA}) \quad (9)$$

the highly solvent accessible (upper boundary) denatured state model ($\Delta G^\circ_{tr\,ub}$) and the compact (lower boundary) denatured state model ($\Delta G^\circ_{tr\,lb}$) from water to osmolyte solution ***(28)***. In these calculations, Δg°_i and n_i have the same meanings as in **Eq. 7**. However, α_i values are obtained by first taking all side-chain and backbone units as being fully solvent accessible (i.e., α^* as defined above is set to unity), and the mean fractional solvent exposure of side-chain or backbone unit "i" is obtained from $\alpha_i = \alpha^*/n_i$. Finally, (stand ASA) represents the standard accessible surface area of each type of amino-acid side chain or backbone U calculated using a stochastic standard-state model for gly-X-gly[†], while (lower ASA) and (upper ASA) are the lower and upper boundary accessible surface areas of each particular side chain and backbone as given in **Table 1** of Creamer et al. ***(57)***. Thus, transfer Gibbs energies of denatured ensembles that are highly solvent-accessible, those that have low solvent accessibilities (compact denatured ensemble), and those in between are bracketed by $\Delta G^\circ_{tr\,ub}$ and $\Delta G^\circ_{tr\,lb}$.

6. Conclusion

When used with knowledge of its shortcomings, the Transfer Model has been highly successful in providing fundamental under-

[†] Two models are used in these calculations: the extended tripeptide model, gly-X-gly, and the stochastic gly-X-gly model ***(54,55)***.

standing and insight into such basic processes as the hydrophobic effect, urea and guanidine hydrochloride denaturation of proteins, and the stabilization of proteins by naturally occurring osmolytes. This model is particularly helpful in identifying the major component parts of the protein that—through interaction with solvent—result in the folded structure that is the native state. The procedures and bases for transfer Gibbs energy changes are elementary in their conception, and are also steeped in the history of biophysical chemistry.

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