Hydrophobicity Regained

ARTICLE *in* PROTEIN SCIENCE · JUNE 1997

Impact Factor: 2.85 · DOI: 10.1002/pro.5560060618 · Source: PubMed

1 AUTHOR:

Paul Andrew Karplus
Oregon State University
199 PUBLICATIONS **14,825** CITATIONS

SEE PROFILE

Protein Science (1997), *6*:1302–1307. Cambridge University Press. Printed in the USA.

Hydrophobicity regained

P. ANDREW KARPLUS

Section of Biochemistry, Molecular and Cell Biology, Cornell University, Ithaca, New York 14853

(RECEIVED December 10, 1996; ACCEPTED March 6, 1997)

Abstract

A widespread practice is to use free energies of transfer between organic solvents and water ($\Delta G^\circ_{transfer}$) to define hydrophobicity scales for the amino acid side chains. A comparison of four $\Delta G^\circ_{transfer}$ scales reveals that the values for hydrogen-bonding side chains are highly dependent on the non-aqueous environment. This property of polar side chains violates the assumptions underlying the paradigm of equating $\Delta G^\circ_{transfer}$ with hydrophobicity or even with a generic solvation energy that is directly relevant to protein stability and ligand binding energetics. This simple regaining of the original concept of hydrophobicity reveals a flaw in approaches that use $\Delta G^\circ_{transfer}$ values to derive generic estimates of the energetics of the burial of polar groups, and allows the introduction of a "pure" hydrophobicity scale for the amino acid residues.

Keywords: amino acids; atomic solvation parameters; hydrophobic effect; hydrophobicity; protein folding; protein stability

The hydrophobic effect describes the thermodynamics of the partitioning of non-polar compounds between water and a non-aqueous phase. The hallmarks of the hydrophobic effect are that the transfer of non-polar compounds into water at ambient temperature is unfavorable due to a decrease in entropy and is associated with a large increase in heat capacity. Although the molecular details that give rise to the hydrophobic effect are still debated (Blokzijl & Engberts, 1993), this has not hindered it from becoming the dominant paradigm for understanding protein folding and stability (e.g., Kauzmann, 1959; Dill, 1990a; Ponnuswamy, 1993; Rose & Wolfenden, 1993; Pace, 1995).

Much work in this field is based on the concept developed by Tanford and coworkers (Tanford, 1962; Nozaki & Tanford, 1971) that the free energy of transfer of amino acids from organic solvents to water ($\Delta G^\circ_{transfer}$) could be used to establish a hydrophobicity scale that is useful for estimating the contributions that buried residues make to the stability of proteins. Over the years, numerous "hydrophobicity scales" and "solvation parameters" have been proposed based on both theoretical considerations and $\Delta G^\circ_{transfer}$ measurements using a variety of compounds to represent the amino acid side chains and a variety of organic solvents or the vapor phase as the non-aqueous solvent. Differences among such scales have fueled an active debate regarding which values, if any, are the ones that are relevant for protein folding (for a review of various viewpoints related to this concept see section II of Lazaridis et al., 1995), and led some to abandon the paradigm of hydrophobicity in favor of the more absolute concept of hydration (Makhatadze & Privalov, 1995). It has been noted that the debate as framed is really unresolvable because the interior of a protein is heterogeneous so that "one would not expect that any single solvent or physical environment could, except by an occasional coincidence, represent the variety of environments experienced by residues within a protein" (Rose & Wolfenden, 1993). I document here that the concept of equating $\Delta G^\circ_{transfer}$ with hydrophobicity (or solvation free energy) for all amino acid side chains is flawed because it involves an inappropriate application of the paradigm of hydrophobicity to polar (hydrophilic) groups, and that much of the debate about hydrophobicity scales can be resolved by a regaining of the original concept of hydrophobicity.

Reprint requests to: P. Andrew Karplus, Section of Biochemistry, 223 Biotechnology Building, Cornell University, Ithaca, New York 14853; email: pak4@cornell.edu.

Four diverse "hydrophobicity" scales

Four sets of $\Delta G^\circ_{transfer}$ for the amino acid side chains are illustrated in Figure 1. Three of these scales are commonly used hydrophobicity scales and the fourth, which involves the polar solvent N-methyl-acetamide (dielectric constant 191) was generated as an extreme test of how solvent polarity affects $\Delta G^\circ_{transfer}$ (Damodaran & Song, 1986). These four scales include three different kinds of amino acid residue analogs and four different solvents which range in dielectric constant from 2 to 191. A quick glance at the figure reveals the striking observation that the non-polar and weakly polar side chains have similar energetics in all systems, whereas the side chains containing polar atoms tend to be more variable in magnitude and sign.

The consistent results for the non-polar residues confirms the ideas reviewed by Kauzmann (1959) that for these groups the partitioning between water and a variety of non-aqueous solvents reflects a highly robust phenomenon in which the energetics are largely dependent on the removal from water and little influenced

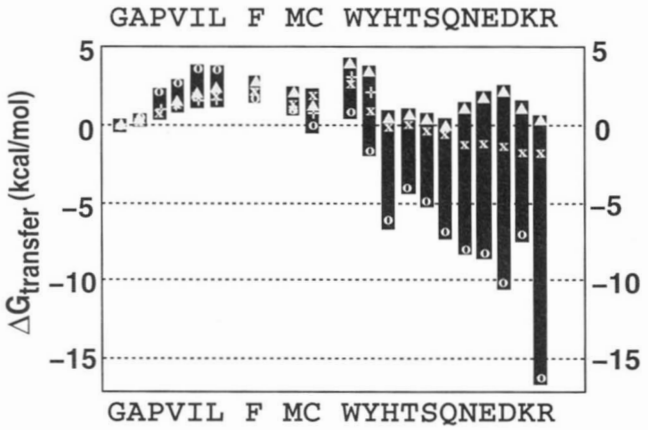

Fig. 1. Comparisons of four experimental $\Delta G^{\circ}_{\text{solvent}\rightarrow\text{water}}$ scales for the amino acid side chains. The four scales shown were based on the solubilities of the free amino acids in ethanol or dioxane (+) (Nozaki & Tanford, 1971), or in N-methyl-acetamide (Δ) (Damodaran & Song, 1986), the partitioning of N-acetyl-amino acid-amides between water and octanol (×) (Fauchere & Pliska, 1983), and the partitioning of side-chain analogs between water and cyclohexane (○) (Radzicka & Wolfenden, 1988). For each amino acid type, the variation in $\Delta G^{\circ}_{\text{transfer}}$ is highlighted by the shaded bar. The residues are divided into four groups from left to right based on side-chain characteristics: Aliphatic non-hydrogen-bonding, aromatic non-hydrogen-bonding, sulfur-containing, and hydrogen-bonding.

by any specific interactions that might occur in the target solvent. A weak dependence on the target environment exists, as the $\Delta G^{\circ}_{\text{transfer}}$ values for the purely aliphatic side chains (Ala, Pro, Val, Ile, Leu) are roughly 50–75% larger for cyclohexane than for octanol. This variation is due to the combined effects of at least three factors of unknown relative importance: octanol contains significant dissolved water (Radzicka & Wolfenden, 1988), octanol is somewhat polar, and side chain analogs have different solvation properties due to the absence of the polar main-chain atoms. Which values are most relevant for protein folding depends on the relative importance of these factors. In any case, the purely aliphatic side chains have the highest $\Delta G^{\circ}_{\text{transfer}}$ values in cyclohexane, whereas for the aromatic (Phe) and the sulfur containing side chains (Met, Cys), cyclohexane gives the lowest values. This is consistent with a polarity scale with aliphatic hydrocarbon < aromatic hydrocarbon < sulfur, and with the assessment that sulfur is a poor hydrogen bond participant.

In contrast, for the side chains which have one or more hydrogen bonding atoms, the $\Delta G^{\circ}_{\text{transfer}}$ values diverge, with significantly positive values in N-methyl-acetamide to large negative values in cyclohexane. The greatest divergence is seen for the side chains capable of forming multiple hydrogen bonds. This indicates that for these residues the non-aqueous solvent and/or the amino acid analog have a large effect on the transfer energetics. The likely explanation for this scatter is that when polar groups are removed from water, they lose favorable hydrogen-bonded interactions with water. Cyclohexane makes no significant polar interactions (Radzicka & Wolfenden, 1988), so the energetics of transfer to cyclohexane will reflect the sum of the favorable transfer of the non-polar side chain portions out of water minus the lost hydrogen bonding interactions with water. The $\Delta G^{\circ}_{\text{transfer}}$ values for the other solvent systems must reflect a similar favorable energy of transfer for the non-polar side chain portions, plus varying amounts of stabilization of the polar side chain portion through a variety of interactions. These interactions include intramolecular hydrogen bonding (Roseman, 1988), and hydrogen bonding with the polar groups of the solvent or residual water that may be dissolved in the solvent. Independent of the relative contributions of these factors, it is clear that the $\Delta G^{\circ}_{\text{transfer}}$ values for polar side chains depend highly on the environment to which they are transferred!

Regaining the original criteria for equating $\Delta G^{\circ}_{transfer}$ and hydrophobicity

The conceptual foundation for the establishment of a $\Delta G^{\circ}_{\text{transfer}}$-based hydrophobicity scale for the amino acids was clearly outlined by Nozaki and Tanford (1971):

> It has been suggested that 100% ethanol can serve as a 'model' for the inside of a protein molecule . . . , i.e., that the difference in free energy between a hydrophobic moiety in 100% ethanol and in water is simply a measure of the unfavorable free energy of interaction with water, non-specific (van der Waals) interactions having about the same free energy in 100% ethanol as in the interior of a protein molecule. Strong support for this idea comes from the finding that the free energy of solvent contacts from one amino acid side chain (norleucine) differs very little between methanol, ethanol, butanol, and acetone, in contrast to the large difference between any one of these solvents and water. . . .
>
> The forgoing principle cannot of course be applied to molecular moieties containing polar groups. Such groups are likely to have specific interactions with hydroxyl, carbonyl, and other polar groups of their environment, and, hence, unlikely to have similar free energies in say, ethanol, dioxane, and the inside of a protein molecule. . . .
>
> For those amino acids which are predominantly hydrophobic (i.e., have similar contact free energies in a variety of media other than water, and a large positive contact free energy in water, relative to all such media) the difference between solvation free energies in water and in 100% ethanol or other non-aqueous media may be considered as establishing a *hydrophobicity scale*.

The parenthetical portion of the last paragraph clearly states two conditions which must exist for solvent transfer energetics to be equated with hydrophobicity: the $\Delta G^{\circ}_{\text{transfer}}$ values must favor the nonaqueous solvent and they must be largely independent of the nonaqueous solvent used. Unfortunately, 25 years of neglect of these assumptions was begun by Nozaki and Tanford themselves as they included the hydrogen bonding side chains Trp, Tyr, His, Thr, and Ser in their "hydrophobicity scale." The inclusion of Trp and Tyr might be understood, because they appeared to fulfill both criteria using dioxane and ethanol as reference solvents, but His, Thr, and especially Ser certainly did not fulfill the second criterion.

Hydrophobicity = $\Delta G^{\circ}_{transfer}$ only for non-polar groups

Based on the above insights it becomes clear that much debate about hydrophobicity is due to a neglect of the two conditions outlined above. Put simply, equating $\Delta G^{\circ}_{\text{transfer}}$ with hydrophobicity (or solvation free energy) only makes sense for moieties which truly are non-polar and it is a misapplication of the paradigm to try to measure or discuss a generic "hydrophobicity" of a polar group. Such groups simply are not hydrophobic; they do not fit the paradigm. In this sense, trying to define the "hydrophobicity" of a

hydrogen-bonding side chain (e.g., arginine) is like trying to use a single color to describe an object which is half blue and half red. Whereas arguments may be made for answers of blue, red, and purple, the only truly defensible answer is "No, the question is not appropriate; the object is not a single color!" Similarly, for the question of what is the free energy associated with the removal of a polar group from water, the only truly justifiable answer is, "There is no generally valid answer; it depends on the environment into which it is transferred." This distinction is also supported by the observation that the enthalpy, entropy, and heat capacity changes associated with the transfer of polar groups to water do not match those associated with the hydrophobic effect.

Measured $\Delta G^{\circ}_{transfer}$ values for amphipathic and polar groups are still highly useful for giving insight into the denaturing or stabilizing properties of various solvents relative to water (e.g., Liu & Bolen, 1995; Tanford, 1964) because they accurately indicate how much better or worse the group in question interacts with the solvent as opposed to water; in this case, the residue's environment inside the folded protein is not relevant. However, suggesting that these $\Delta G^{\circ}_{transfer}$ values have direct relevance for the energetics of protein folding or stability is unreasonable because for polar groups the ΔG° values for burial are highly dependent on the final environment, and each buried group is in a unique environment in the heterogeneous interior of a protein.

An important implication of this analysis is that the conceptual foundation of atomic solvation parameters (Eisenberg & McLachlan, 1986) is flawed because all such parameter sets that include values for polar atoms rest on the invalid assumption that there exists a unique set of $\Delta G^{\circ}_{transfer}$ values that represent the energetics of the burial of polar groups in a protein's interior. Consistent with this insight, two recent reports showed large discrepancies exist between results using atomic solvation parameter sets based on various $\Delta G^{\circ}_{transfer}$ values (Cummings et al., 1995; Juffer et al., 1995), and others have shown that the use of atomic solvation parameters often does not contribute to the ability to accurately estimate the energetics of ligand binding or protein association (Horton & Lewis, 1992; Janin, 1995). This insight also gives a reason for the well-documented breakdown of the surface area proportionality for polar groups (Lazaridis et al., 1995).

A suggested approach

This regaining of the original concept of hydrophobicity makes it clear that using $\Delta G^{\circ}_{transfer}$ values to estimate a generic solvation contribution to the energetics of burial is only valid when dealing with non-polar groups. As was well documented in the early 1970s and applied extensively since then, the hydrophobicity of hydrocarbon groups is related to accessible surface area with a coefficient of roughly 21–24 cal/Å^2 for aliphatic groups and 16–19 cal/Å^2 for aromatic groups (Hermann, 1972; Chothia, 1974; Reynolds et al., 1974). For the aliphatic side chains, the range of $\Delta G^{\circ}_{transfer}$ values seen between the various solvents shown in Figure 1 corresponds to a range of ~18–32 cal/Å^2 non-polar accessible surface area (Fig. 2). (Corrections suggested by Sharp et al. (1991a, 1991b) roughly double these values but the validity of the suggested corrections is still being debated (Ben-Naim, 1994; Honig & Yang, 1995; Lee, 1995)). Since the middle of this range (25 cal/Å^2) is close to the estimates based on pure hydrocarbons, it seems parsimonious to use that value as an estimate of the hydrophobic effect and accept that there is an uncertainty of roughly ±30% (Fig. 2). (This recalls the well-known value of 24 cal/Å^2 value that was suggested by Chothia (1974) but inappropriately applied to both polar and non-polar surface areas based on the explicit, but often overlooked, assumption that the burial of polar atoms in proteins will contribute 1 kcal/mol more stabilization energy than is suggested by the $\Delta G^{\circ}_{transfer}$ values measured for ethanol.)

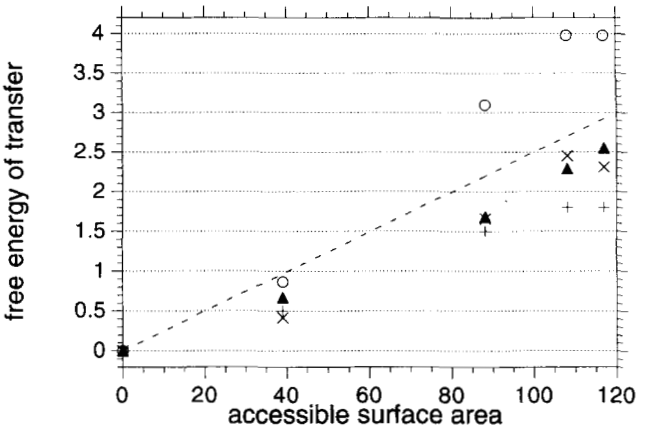

Fig. 2. $\Delta G^{\circ}_{transfer}$ vs. net side-chain surface area for the four experimental systems shown in Fig. 1 (symbols as in Fig. 1). Only the five purely aliphatic residues are included, with areas taken from Lesser & Rose (1990): Gly (0 Å^2), Ala (39 Å^2), Val (88 Å^2), Ile (108 Å^2), and Leu (117 Å^2). The dashed line represents a compromise value for the relationship between $\Delta G^{\circ}_{transfer}$ and surface area and has a slope of 25 cal/Å^2. Based on the aromatic and aliphatic surface area of Phe, it can be calculated that a value of 16 cal/Å^2 is a consensus value between cyclohexane and octanol for the accessible surface area dependence of aromatic hydrocarbon surface. Both of these values agree reasonably well with studies of pure hydrocarbons, which have shown aromatic surface to be less hydrophobic than aliphatic surfaces (Reynolds et al., 1974). Due to the spread of these measurements there is some uncertainty associated with the magnitude of the hydrophobic effect in protein folding but there need be no uncertainty that it is large and favorable. Two recent reports (Vajda et al., 1995; Chan & Dill, 1997) make a strong case that a value of near 30–34 cal/Å^2 (close to that given by the cyclohexane data) is the best value but, until there is a better consensus, the value of 25 ± 30% has the advantage of effectively covering the ranges which are generally considered as plausible. A range of 33 ± 30% does include the low end possibilities but also includes estimates much higher than are generally considered likely.

For the polar parts of amino acid residues, the energetics associated with burial depends heavily on the details of the final environment. Thus, the energetics cannot be estimated by a generic "hydrophobic effect" approach but must be accounted for by a comparison of the final hydrogen-bonding environment with the lost hydrogen bonding with water: Polar groups buried in a poor hydrogen-bonding environment will have very unfavorable energetics (like the cyclohexane $\Delta G^{\circ}_{transfer}$ values) and those in perfectly complementary hydrogen bonding environments may make large favorable contributions to stability. Whereas the large amount of polar group (especially peptide) burial during protein folding means their energetics are very important (Liu & Bolen, 1995), it seems that estimates of the contribution of polar groups to protein stability can only be made on an individual basis, with knowledge of the final environment. Even then, such estimates will be limited by the accuracy with which polar interaction energies can be calculated (Lazaridis et al., 1995). It should be noted that an additional complexity occurring for polar groups is that they do not even have a generic interaction with water! Multiply hydrogen-bonding substances can have significantly perturbed hydration,

which contributes significantly to transfer energetics (Lemieux, 1996) and clearly violates additivity principles (Dill, 1997).

In the face of these considerations, a reasonable approach is to ignore the polar atoms when accounting for the *generic* contributions of group burial to stability (this is equivalent to assigning polar atoms a value of zero in terms of atomic solvation parameters), and in this way clearly separate the hydrophobic and the environment dependent contributions to protein stability. Based on this simplification, a table can be constructed giving an estimate of the hydrophobic effect expected to be associated with the burial of each complete side chain (Table 1). For non-polar side chains, the reported values are similar to the commonly used values based on $\Delta G^{\circ}_{transfer}$. However, for the polar residues, the reported values differ significantly. A practical application of the values in this table is for properly estimating the contribution of the hydrophobic effect to the change in stability of mutants. For instance, for a mutation of a buried Lys → Ala there is a 2.7 kcal/mol discrepancy in accounting for the contribution of the hydrophobic effect: The new values show a contribution of 1.0 − 1.9 = −0.9 kcal/mol which is *destabilizing*, whereas use of the octanol-based values yields a *stabilizing* contribution of 0.42 − (−1.35) = 1.77 kcal/mol. This clarification is important for proper accounting of the relative contributions of the hydrophobic effect and hydrogen bonding in mutation studies (Pace, 1995).

It is worth emphasizing how the hydrophobicity scale of Table 1 relates to the multitude of hydrophobicity scales which are present in the literature (reviewed in Ponnuswamy, 1993). As noted above, the scale presented in Table 1 is a pure hydrophobicity scale in that it estimates the free energy due to the hydrophobic effect for transferring a residue from water to a non-aqueous solvent. All other scales could be denoted as "hydrophobicity *plus*" scales, as each includes other factors in addition to the hydrophobic effect. Direct $\Delta G^{\circ}_{transfer}$-

Table 1. *Estimated contribution of the hydrophobic effect to the burial of each type of amino acid residue and side chain. This table is similar in concept to that presented by Tanford (1962) in which the hydrophobicity of many polar side chains was estimated by their non-polar atom content rather than measured* $\Delta G^{\circ}_{transfer}$ *values*

Residue type	Residue non-polar surface area[a] (Å^2)	Estimated hydrophobic effect for residue burial[b] (kcal/mol)	Estimated hydrophobic effect for side chain burial[c] (kcal/mol)	$\Delta G^{\circ}_{transfer}$ octanol[d] (kcal/mol)
Gly	47	1.18	0.0*	0.0*
Ala	86	2.15	1.0	0.42
Val	135	3.38	2.2	1.66
Ile	155	3.88	2.7	2.45
Leu	164	4.10	2.9	2.31
Pro	124	3.10	1.9	0.98
Cys	48	1.20	0.0	2.09
Met	137	3.43	2.3	1.67
Phe	39 + 155	3.46	2.3	2.43
Trp	37 + 199	4.11	2.9	3.06
Tyr	38 + 116	2.81	1.6	1.31
His	43 + 86	2.45	1.3	0.18
Thr	90	2.25	1.1	0.35
Ser	56	1.40	0.2	−0.05
Gln	66	1.65	0.5	−0.30
Asn	42	1.05	−0.1	−0.82
Glu	69	1.73	0.5	−0.87
Asp	45	1.13	−0.1	−1.05
Lys	122	3.05	1.9	−1.35
Arg	89	2.23	1.1	−1.37

[a]The hydrocarbon surface areas for the residues were summed from the values given for individual atoms by Lesser and Rose (1990). All surfaces associated with main- and side-chain carbon atoms were included except for amide, carboxylate, and guanidino carbons (main-chain C, Cγ of Asp, Asn, Cδ of Glu, Gln, Cζ of Arg). For aromatic side chains, the aliphatic and aromatic surface areas are reported separately. Although the sulfur atoms of Cys and Met may have some truly hydrophobic character, this is not accounted for here.

[b]The magnitude of the hydrophobic effect is assigned to be proportional to apolar surface area alone with coefficients of 25 cal/Å^2 for aliphatic carbon atoms and 16 cal/Å^2 for aromatic carbon atoms (see Fig. 2 legend for the rationale). The true magnitude of the hydrophobic effect is expected to be within ±30% of the value in the table. No account is made for the polar atoms because their contribution to stability is not governed by the hydrophobic effect. This scale is still an oversimplification because it does not account for variations in hydrophobicity for carbon atoms bonded to or near in space to polar atoms. Unfortunately, true values for these atoms may not be measurable, because the context dependency of the $\Delta G^{\circ}_{transfer}$ values of polar atoms leads to an ambiguity in separating the effects of the polar atom itself from those on the hydrocarbon to which it is adjacent. Although the magnitude is unknown, such considerations would decrease the hydrophobicities of Gly, Ala, Pro, and all the hydrogen-bonding side chains.

[c]The values are obtained from the previous column by subtracting the value for Gly (1.18 kcal/mol) from each residue. The errors in these values may, of course, be much larger than 30%. The asterisk denotes that the value for Gly is defined as zero in this and the following scale.

[d]The values are taken from Fauchere & Pliska (1983), and are probably the most commonly used values for estimating the contribution of the hydrophobic effect to changes in stability of mutants. It should be noted that some publications (Creighton, 1994; Ponnuswamy, 1993) of the list mistakenly report the Π-values, and these are a factor of 1.36 smaller than the free energies in kcal/mol.

based scales include the hydrophobic effect *plus* how well the hydrogen bonding groups are satisfied by the given non-aqueous solvent. Scales based on how frequently residues are buried in proteins include the hydrophobic effect *plus* factors related to protein structure and evolution. For instance, it is well known that proline residues are buried much less frequently than their chemical hydrophobicity would predict (Ponnuswamy, 1993). As has been noted by Lesser and Rose (1990), this is presumably because steric and hydrogen-bonding restraints override hydrophobicity considerations in determining proline placement in proteins. Recognizing the extra factors present in every "hydrophobicity *plus*" scale allows an understanding of why correlations between such scales are far from perfect. The pure hydrophobicity scale is not better than "hydrophobicity *plus*" scales, just different. Indeed, whereas the pure hydrophobicity scale is useful for estimating the free energy contribution of the hydrophobic effect, the "hydrophobicity *plus*" scales are each useful for predictions and analyses related to their origin. For instance, a burial-based "hydrophobicity *plus*" scale is best for predicting whether or not residues will be buried in a protein.

Outlook

As described by Dill (1990b), the true hydrophobic contribution takes into account a complete transfer process involving "(a) removal of the solute from the pure medium, with the breaking of solute-solvent bonds, (b) closing the cavity therein, (c) creating a cavity in water, and (d) making the solute water bonds." The hydrophobic effect contribution to stability does not take into account any environment-dependent effects, such as van der Waals and other non-covalent interactions in a specific environment in a protein, which may be stronger (or weaker) than the non-bonded interactions present in a generic non-aqueous solvent. Mutagenesis studies show that such "packing effects" contribute significantly to protein stability (e.g., Eriksson et al., 1992) but, as with the polar interactions, these are highly environment dependent and cannot be assessed in a generic way. It is also possible to place the energetics of polar group burial in a similar framework: The polar groups can be assigned a generic contribution to stability of 0 kcal/mol, with the actual contribution of each group deviating from zero due to "packing effects" which relate to whether its hydrogen-bonding environment is more or less favorable than its interactions with water.

This return to the original concepts related to the quantification of hydrophobicity by using $\Delta G^{\circ}_{\text{transfer}}$ values should allow the field to move beyond questions such as "Which solvent system is the best model for the interior of a protein?" The answer is that for polar groups, no solvent can effectively mimic the heterogeneous interior of a protein, but for non-polar groups the phenomenon of hydrophobicity is remarkably robust so that all non-aqueous solvents can be said to be effective mimics, with all amino acid analogs and solvent systems yielding driving forces within 30% of the values obtained in experiments on hydrocarbons. A valid area for continued research is to more precisely define where in this range the best value for the generic contribution of non-polar amino acid side chains lies (Vajda et al., 1995; Chan & Dill, 1997). Nevertheless, it should be noted that an accuracy of 30% already compares favorably with current abilities to estimate other major contributions to protein stability such as conformational entropy and hydrogen bonding. With this regained formulation, the hydrophobic effect always contributes favorably and significantly to protein stability, and it may even be the part of the protein stability equation that can be most accurately estimated.

Acknowledgments

I thank Shing Ho and Victor Shu for fruitful discussions, Ken Dill, Harold Scheraga, and my research group for comments on the manuscript, and the Oregon State University Department of Biochemistry and Biophysics for hosting my sabbatical during which these ideas were formulated. This work was partially supported by a fellowship from the John Simon Guggenheim Memorial Foundation.

References

Ben-Naim A. 1994. Solvation: From small to macro molecules. *Curr Op Struct Biol 4*:264–268.

Blokzijl W, Engberts JBFN. 1993. Hydrophobic effects. Opinions and facts. *Angew Chem Int Ed Engl 32*:1545–1579.

Chan HS, Dill KA. 1997. Solvation: How to obtain microscopic energies from partitioning and solvation experiments. *Ann Rev Biophys Biomolec Struct 26*. Forthcoming.

Chothia C. 1974. Hydrophobic bonding and accessible surface area in proteins. *Nature 248*:338–339.

Creighton T. 1994. *Proteins: Structures and molecular properties*. New York: Freeman Press.

Cummings MD, Hart TN, Read RJ. 1995. Atomic solvation parameters in the analysis of protein–protein docking results. *Protein Sci 4*:2087–2099.

Damodaran S, Song KB. 1986. The role of solvent polarity in the free energy of transfer of amino acid side chains from water to organic solvents. *J Biol Chem 261*:7220–7222.

Dill KA. 1990a. Dominant forces in protein folding. *Biochemistry 29*:7133–7155.

Dill KA. 1990b. The meaning of hydrophobicity. *Science 250*:297.

Dill KA. 1997. Additivity principles in biochemistry. *J Biol Chem 272*:701–704.

Eisenberg D, McLachlan AD. 1986. Solvation energy in protein folding and binding. *Nature 319*:199–203.

Eriksson AE, Baase WA, Zhang X-J, Heinz DW, Blaber M, Baldwin EP, Matthews BW. 1992. Response of a protein structure to cavity-creating mutations and its relation to the hydrophobic effect. *Science 255*:178–183.

Fauchere J-L, Pliska V. 1983. Hydrophobic parameters π of amino acid side chains from the partitioning of N-acetyl-amino-acid amides. *Eur J Med Chem 18*:369–375.

Hermann RB. 1972. Theory of hydrophobic bonding. II. The correlation of hydrocarbon solubility in water with solvent cavity surface area. *J Phys Chem 76*:2754–2759.

Honig B, Yang A-S. 1995. Free energy balance in protein folding. *Adv Prot Chem 46*:27–55.

Horton N, Lewis M. 1992. Calculation of the free energy of association for protein complexes. *Protein Sci 1*:169–181.

Janin J. 1995. Elusive Affinities. *Proteins Struct Funct and Genet 21*:30–39.

Juffer AH, Eisenhaber F, Hubbard SJ, Walther D, Argos P. 1995. Comparison of atomic solvation parametric sets: Applicability and limitations in protein folding and binding. *Protein Sci 4*:2499–2509.

Kauzmann W. 1959. Some factors in the interpretation of protein denaturation. *Adv Prot Chem 14*:1–63.

Lazaridis T, Archontis G, Karplus M. 1995. Enthalpic contribution to protein stability: Insights from atom-based calculations and statistical mechanics. *Adv Prot Chem 47*:231–296.

Lee B. 1995. Analyzing solvent reorganization and hydrophobicity. *Meth Enzymol 259*:555–576.

Lemieux RU. 1996. How water provides the impetus for molecular recognition in aqueous solution. *Acc Chem Res 29*:373–380.

Lesser GJ, Rose GD. 1990. Hydrophobicity of amino acid subgroups in proteins. *Proteins Struct Funct Genet 8*:6–13.

Liu Y, Bolen DW. 1995. The peptide backbone plays a dominant role in protein stabilization by naturally occurring osmolytes. *Biochemistry 34*:12884–12891.

Makhatadze GI, Privalov PI. 1995. Energetics of protein structure. *Adv Prot Chem 47*:307–417.

Nozaki Y, Tanford C. 1971. The solubility of amino acids and two glycine peptides in aqueous ethanol and dioxane solutions. *J Biol Chem 246*:2211–2217.

Pace CN. 1995. Evaluating the contribution of hydrogen bonding and hydrophobic bonding to protein folding. *Meth Enzymol 259*:538–554.

Ponnuswamy PK. 1993. Hydrophobic characteristics of folded proteins. *Prog Biophys Molec Biol 59*:57–103.

Radzicka A, Wolfenden R. 1988. Comparing the polarities of the amino acids: side-chain distribution coefficients between the vapor phase, cyclohexane, 1-octanol, and neutral aqueous solution. *Biochemistry 27*:1664–1670.

Reynolds JA, Gilbert DB, Tanford C. 1974. Empirical correlation between hy-

drophobic free energy and aqueous cavity surface area. *Proc Nat Acad Sci USA 71*:2925–2927.

Rose GD, Wolfenden R. 1993. Hydrogen bonding, hydrophobicity, packing, and protein folding. *Ann Rev Biophys Biomol Struct 22*:381–415.

Roseman MA. 1988. Hydrophobicity of polar amino acid side chains is markedly reduced by flanking peptide bonds. *J Mol Biol 200*:513–522.

Sharp KA, Nicholls A, Fine RF, Honig B. 1991a. Reconciling the magnitude of the microscopic and macroscopic hydrophobic effects. *Science 252*:106–109.

Sharp KA, Nicholls A, Freidman R, Honig B. 1991b. Extracting hydrophobic free energies from experimental data: Relationship to protein folding and theoretical models. *Biochemistry 30*:9686–9697.

Tanford C. 1962. Contribution of hydrophobic interactions to the stability of the globular conformation of proteins. *J Am Chem Soc 84*:4240–4247.

Tanford C. 1964. Isothermal unfolding of globular proteins in aqueous urea solutions. *J Am Chem Soc 86*:2050–2059.

Vajda S, Weng Z, DeLisi C. 1995. Extracting hydrophobicity parameters from solute partition and protein mutation/unfolding experiments. *Protein Engineering 8*:1081–1092.