Limited Validity of Group Additivity for the Folding Energetics of the Peptide Group

Franc Avbelj[1]* **and Robert L. Baldwin**[2]*
[1]*National Institute of Chemistry, Ljubljana, Slovenia*
[2]*Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, California*

ABSTRACT **The principle of group additivity is a standard feature of analyses of the energetics of protein folding, but it is known that it may not always be valid for the polar peptide group. The neighboring residue effect shows that group additivity is not strictly valid for a heteropeptide. We show here that group additivity fails seriously for peptide groups close to either peptide end, even for a homopeptide that has blocked end groups with no formal charges involved. The failure of group additivity is caused by the electrostatic character of the solvation of peptide polar groups and is illustrated with values of the electrostatic solvation free energy (ESF) calculated by DelPhi. Solvation free energies and enthalpies are known experimentally for monoamides and are often used to model the solvation of peptide groups, but ESF results show that monoamide values are very different from those of peptide groups. A main cause of the difference is that peptide solvation depends on the dipole–dipole interactions made between adjacent peptide groups, which vary with peptide conformation. Ligands that interact with the peptide backbone by an electrostatic mechanism could show a similar peptide end effect, and hydrogen exchange results from the literature confirm that exchange rates are position-dependent close to peptide ends. Proteins 2006;63:283–289.** © 2005 Wiley-Liss, Inc.

Key words: protein folding energetics; electrostatic solvation; position dependent properties

INTRODUCTION

The principle of group additivity is used commonly to analyze the energetics of protein folding: see the review by Dill[1] of additivity principles in biochemistry. Group additivity states that, if two components A and B contribute independently to some process, then their enthalpies or free energies or entropies are additive. Additivity often works well for enthalpies even in processes where the free energies or entropies are not additive.[1]

Analyses of how peptide H-bonds contribute to the energetics of folding often invoke group additivity by saying that the peptide group (—CONH—) contributes independently of neighboring groups to the enthalpy change for H-bond formation (see Makhatadze and Privalov[2]). If this is valid, then the interactions between water and the free peptide group, which must be broken to make the peptide H-bond, ought to be modeled reliably by an amide such as N-methylacetamide (NMA), which contains the —CONH— group. This proposal is an implicit part of the early test by Klotz and coworkers[3,4] of whether peptide H-bonds are stable in water: they asked if two NMA molecules form H-bonded dimers in water. A main purpose of this paper is to show that monoamides are not satisfactory models for the interaction of the free peptide group with water because the principle of group additivity fails in this case (see also Roseman[5,6]).

Background: Peptide H-bonds and the Energetics of Protein Folding

Before a peptide H-bond can be made, H-bonds between water and the —NH and —C═O groups of the unfolded peptide must be broken. Thus, knowledge of the solvation of the free peptide group is critical for understanding the energetic role of the peptide group in protein folding. Efforts to analyze this role simply by counting H-bonds have proven illusory.[7] If water (W) makes two H-bonds to the free peptide group, one to the peptide —NH moiety and another to the peptide —C═O moiety, while the —NH and —C═O moieties make only one H-bond in forming a peptide H-bond, then H-bonds apparently oppose folding by 2:1 because the W···HN—, W···O═C— and —NH···O═C— H-bonds all have approximately equal gas phase energies, -6 ± 1 kcal/mol.[8] But there is a paradox: the alanine peptide helix is stable in water,[9] and helix formation is enthalpy driven. Moreover, the helix enthalpy change is produced by H-bonds made by peptide groups, not by burial of nonpolar surface area, because ΔCp is too small to be measurable,[10–12] in contrast to burial of nonpolar surface area.

A possible solution to the H-bond paradox is given by the H-bond inventory,[13] according to which W···W H-bonds are formed stoichiometrically with peptide H-bonds, as indicated in the equation:

Grant sponsor: Ministry of Education and Science of Slovenia.

R.L.B. dedicates this paper to George Rose, a valued friend and a pioneer in studying the mechanism of protein folding.

*Correspondence to: Franc Avbelj, National Institute of Chemistry, Hajdrihova 19, Ljubljana Sl 1115, Slovenia. E-mail: francl@sg3.ki.si or Robert L. Baldwin, Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, CA 94305-5307. E-mail: bbaldwin@cmgm.stanford.edu

Received 12 May 2005; Revised 11 July 2005; Accepted 12 July 2005

Published online 15 November 2005 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/prot.20756

$$W\cdots HN\text{—} + W\cdots O{=}C\text{—} = \text{—}NH\cdots O{=}C\text{—} + W\cdots W \quad (1)$$

The H-bond inventory predicts that two H-bonds are present in both reactants and products, so that ΔH(predicted) = 0 ± 1 kcal/mol for the alanine peptide helix, in agreement with the observed value of −0.9 kcal/mol per residue.[10–12]

The H-bond inventory gives the wrong answer, however, for amide solvation.[7] It predicts a net change of only one H-bond when an isolated amide molecule (in the gas phase) is solvated in liquid water, although the known enthalpy change for solvation (~ −12 kcal/mol) predicts a net change of two H-bonds. Thus, W···W H-bonds are probably not formed stoichiometrically with peptide H-bonds. The failure of the H-bond inventory might be explained by the large excess of water (55 M) over amide (~1 mM) in amide solvation experiments and the likelihood that some liquid water molecules have broken H-bonds.[14] A more basic reason for the failure of the H-bond inventory is given by the electrostatic approach to polar solvation, which indicates that the H-bond inventory is too simplified to be correct. The electrostatic approach provides a framework for predicting correctly the enthalpy changes for both amide solvation and peptide helix formation: see below.

METHODS

ESF and E_{local} (at a given site) are calculated as described by Avbelj[15] and Avbelj and Baldwin.[16,17] E_{local} is calculated from Coulomb's law with a dielectric constant of 1 and with point charges assigned to the main-chain atoms N, H_N, C, and O in two adjacent peptide groups as described.[15] ESF is the backbone ESF with side-chain partial charges set = 0. It is calculated by the finite difference Poisson-Boltzmann method,[15,18] using the PARSE parameter set.[18]

RESULTS AND DISCUSSION

Solvation of the Polar Peptide Group Analyzed by an Electrostatic Approach

For years, solution chemists have analyzed the solvation of polar small molecules by an electrostatic approach, whose adoption undoubtedly traces to the remarkable success of the Born equation[19] in explaining the huge solvation free energies and enthalpies of monovalent anions and cations. Experimental measurements of ion solvation were motivated by the Born equation (1920) and came after 1920. The Born equation considers the work of charging a spherical ion in vacuum and in liquid water, and the solvation free energy of the ion is taken to be the difference between the two charging processes; water is treated as a continuum solvent.

$$-\Delta G^{o} = (q^2/2r)[1 - (1/D)] \quad (2)$$

Here q is the charge, r is the radius of the ion, and D is the dielectric constant of water. Uncertainty concerning the correct value of the ion radius r[20,21] prevents the Born equation from being entirely successful at predicting ion solvation free energies a priori. When the dielectric constant of the solvent is large, as is the case for water (D = 78.5 at 25°C), the Born equation predicts that the solvation free energy is almost entirely enthalpic.[20] Figure 1 compares the observed ΔG° and ΔH values for solvation of some common monovalent anions and cations.[22] The figure shows that ΔG° varies linearly with ΔH and the two are nearly equal numerically. The Born equation predicts that ΔH is proportional to ΔG° with a proportionality factor of 1.017 when the dielectric constant is 78.5 [see Equation (8) in Rashin and Honig[20]]. When the origin (0,0) is included in the plot of experimental solvation enthalpy versus solvation free energy for ions (see Fig. 1), the proportionality factor of ΔH to ΔG° is 1.072 and the correlation coefficient is 0.999.

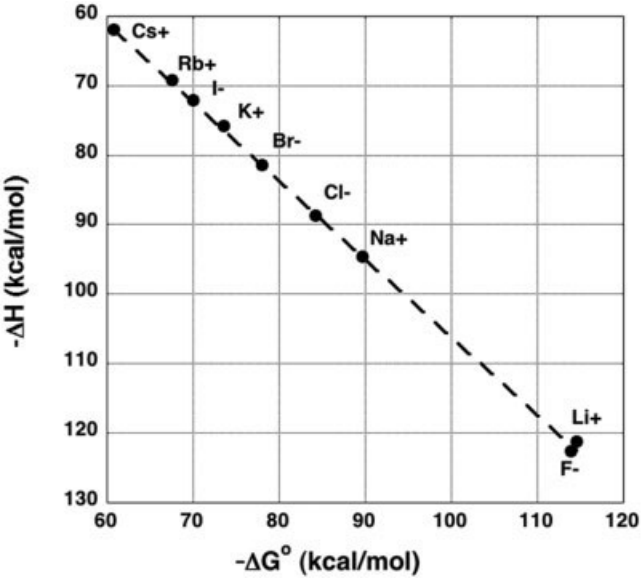

Fig. 1. Ion solvation enthalpy plotted against ion solvation free energy: data from Robinson and Stokes.[22] The free energy of ion solvation is almost entirely enthalpic and ΔH is linearly related to ΔG°.

The free energy of amide solvation, like that of ion solvation, is almost entirely enthalpic.[23] Amide solvation free energies are much smaller than those of monovalent ions and experimental errors are relatively larger: thus ΔG° is nearly equal to ΔH, apparently within error, for four different amides (Fig. 2). Near equivalence of solvation free energy and enthalpy may turn out to be a general property of polar solvation in water, as predicted by the Born equation. Amide solvation free energies were first measured in pioneering work by Wolfenden,[24] who used isotopic labeling to detect the minute quantity of amide present in the gas phase in equilibrium with amide in aqueous solution. Enthalpies of solvating amides were measured especially by Della Gatta and coworkers,[25] who combined calorimetrically measured heats of vaporization and solution. Entropies of solvation are small compared to the free energy changes both for amides and for monovalent ions (see also Dunitz[26]), which may reflect the fact that water molecules in liquid water are part of a random

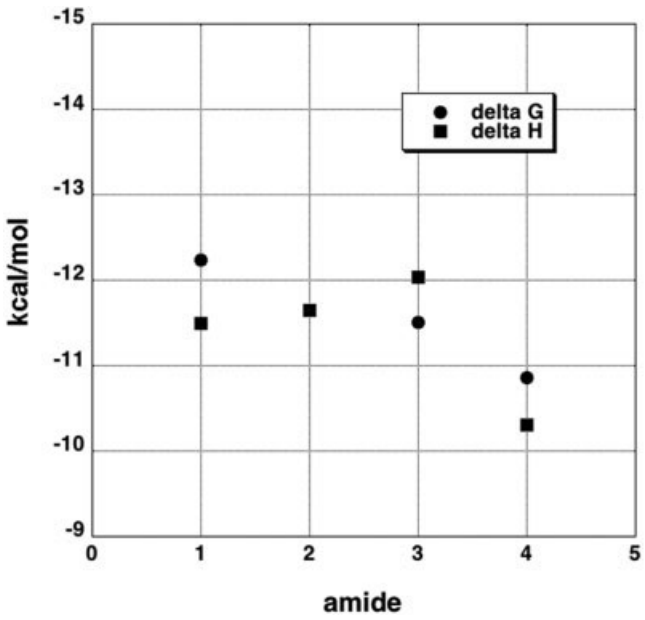

Fig. 2. The free energy and enthalpy of amide polar solvation are compared for 4 different amides: 1 = N-methylacetamide, 2 = acetamide, 3 = propionamide, 4 = N,N-dimethylacetamide. The free energy is almost equal to the enthalpy. Data are from Avbelj et al.[23]; see also Wolfenden,[24] Della Gatta et al.[25]

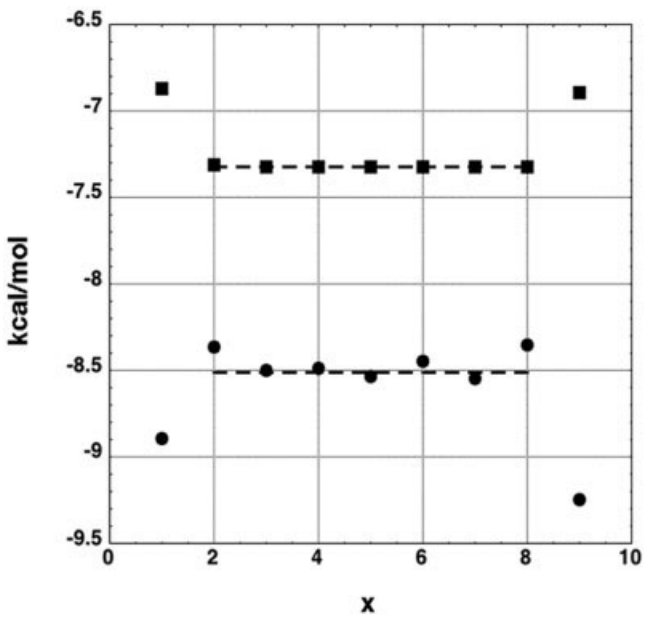

Fig. 3. Values of ESF (■) and E_{local} (■) are plotted against residue position (x) for the peptide groups in the peptide $CH_3CO(Ala)_9NHCH_3$. In order to plot both ESF and E_{local} on the same graph, −4 is added to the values of E_{local}. Both ESF and E_{local} are approximately constant at all interior positions, residues 2 − 8, but change substantially, and in an opposite manner, at the terminal residues 1 and 9. The lines show that both variables are approximately constant for x = 2 − 8.

network of H-bonds, and solvation is a process in which water molecules change from one bound state to another. Because solvation of polar groups in water can be understood to a good first approximation by an electrostatic approach, with water treated as a continuum solvent, electrostatic analyses can be employed to understand the properties of polar solvation (see Sitkoff et al.[18]; Florian & Warshel[27]).

Here we use the electrostatic algorithm DelPhi, because the PARSE parameter set of DelPhi has been calibrated against experimental solvation free energies of polar groups in small molecules, including amides.[18] A cavity is carved out of the solvent to contain the peptide or other solute, a finely spaced cubical lattice is placed in the center of the molecule, and the partial charges of the atoms of the solute are set on the grid by using a geometrical construction. Then a finite difference solution to Poisson's equation is obtained. The atomic structure of the solute must be known accurately, but no other adjustable parameters are used.

To obtain the experimental value for the electrostatic free energy in a small molecule study, one must subtract two other terms that contribute to the experimental solvation free energy (and also to the enthalpy). These two terms are the work of making a cavity in water for the solute and the van der Waals interactions between solute and solvent. These two terms are estimated (approximately) by using experimental solvation free energies of nonpolar solutes (alkanes), evaluated at the same solvent-accessible surface area as the solute.[18]

Electrostatic solvation of a peptide group varies both with the accessibility of water to the peptide group and with the peptide dipole–dipole interactions, which depend strongly on backbone conformation. The dipole–dipole interactions are those made by a pair of adjacent peptide groups[28] on either side of C_α; they are expressed quantitatively by the local electrostatic energy, E_{local},[28] which is computed by Coulomb's law using atom-centered partial charges (see Methods). Three energy terms determine whether or not a given backbone conformation is populated according to the electrostatic screening model (ESM[16]), which is based on a proposal by Avbelj and Moult.[28] The three energy terms are: E_{local},[28] ESF, and the torsional potentials $V(\phi)$ and $V(\psi)$. The relative orientation of the two dipole moments on the pair of adjacent peptide groups determines how favorable the dipole–dipole interaction is.[29] Wada[30] measured the apparent peptide dipole moment of the trans peptide group as 3.4 Debye units per residue by determining how the overall dipole moment of an α-helix depends on its degree of polymerization for helical poly-γ-benzyl-L-glutamate in ethylene dichloride. Dipoles on adjacent peptide groups are anti-parallel in the extended-β conformation (−120°, 120°), which gives a favorable value of E_{local}, while the peptide dipoles are parallel in the α_R conformation (−60°, −40°), which gives an unfavorable value of E_{local}. Peptide H-bonds are needed to stabilize the α_R conformation according to the ESM. Although E_{local} can assume either negative (favorable) or positive (unfavorable) values, ESF is always negative (favorable).[15]

ESF Values of Monoamides Compared With Peptide Groups

Figure 3 shows ESF values of peptide groups in a nine-residue acetyl-alanine-N-methylamide peptide

($CH_3CO(Ala)_nNHCH_3$); n = 9) in the extended-β conformation (−120°, 120°). Interior peptide groups (residues 3–7) have constant ESF values (−8.51 ± 0.03 kcal/mol) while the N-terminal (−8.89 kcal/mol) and C-terminal (−9.25 kcal/mol) peptide groups have larger −ESF values. (The ESF value given here is a larger negative number than the one given previously[23] because the earlier value was obtained when studying Ala to Val substitutions and certain backbone geometric parameters are different for Ala and Val.) Figure 4 gives the average of the N— and C— terminal ESF values for peptides with chain lengths n = 2, 3, . . .9 and also gives the peptide ESF value for n = 1. The ESF value for n = 0 is that of N-methylacetamide. Figure 4 further compares the ESF results with the behavior of E_{local}. There is a sharp drop in −ESF between n = 0 and 1 and a smaller drop between n = 1 and 2; then the terminal ESF value remains almost constant for n = 2 and higher. E_{local} shows the inverse behavior: $-E_{local}$ rises sharply between n = 0 and 1, rises again between n = 1 and 2, and then remains constant. The E_{local} value for n = 0 is that of NMA. E_{local} contains an arbitrary constant, which is set equal to 0 for NMA; −4 has been added to E_{local} in Figures 3 and 4 in order to show ESF and E_{local} on one graph. There are two —NHCO— groups in the n = 1 alanine peptide: in the residue numbering scheme used in Figure 3, one peptide group is assigned to NMA and the other is assigned to the single alanine residue.

The inverse variation of ESF and E_{local} in Figures 3 and 4 is typical of the behavior of these two variables. For example, ESF and E_{local} also vary in an inverse manner as the backbone conformation changes.[15] Because both ESF and E_{local} depend on the dipole–dipole interactions between adjacent peptide groups and ESF depends also on the accessibility of water to the peptide polar groups,[15] it is not surprising that NMA, which has only one —NHCO— group, has very different values of ESF and E_{local} from those of an interior peptide group. Note that the four different amides in Figure 2 have fairly similar values of the experimental polar solvation free energy (which is fitted closely by the computed ESF[18,23]), in contrast to the large differences in ESF between monoamides and peptide groups (Figs. 3, 4).

The contrast in ESF values between NMA and the peptide group (Fig. 4) means that monoamides are unsatisfactory models for the solvation of peptide groups when protein folding thermodynamics are analyzed by a procedure like that of Makhatadze and Privalov.[2] The polar solvation of the peptide group makes a major contribution to the folding energetics. Based on solvation enthalpy data for six different amides, Makhatadze and Privalov[2] give the enthalpy of hydration of the peptide group as −14.2 kcal/mol (their Table 2), a value almost twice as large as the ESF values of peptide groups in Figure 3. They use this number in deriving the enthalpy of the peptide H-bond from unfolding enthalpies for four different proteins and their H-bond enthalpy values range from −9.1 to −14.3 kcal/mol (their Table 10). These values may be compared with the gas phase energy of the peptide H-bond, given as

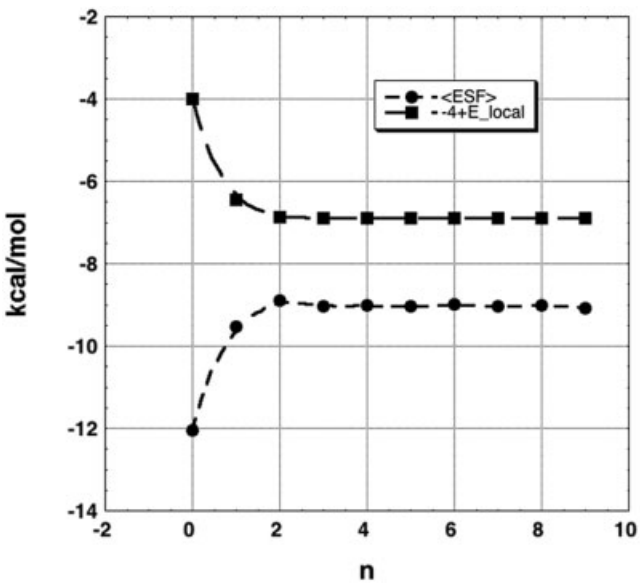

Fig. 4. Values of ESF and E_{local} for the terminal peptide groups in a set of alanine peptides, $CH_3CO(Ala)_nNHCH_3$, are plotted against n, the number of alanine residues. The ESF and E_{local} values for n = 0 are those of N-methylacetamide. For n = 2, 3…9, the average ESF or E_{local} of the N- and C-terminal peptide groups is shown. For n = 1, the single terminal peptide group is shown. The ESF and E_{local} values are approximately constant for n = 2–9 but they vary inversely to each other for n between 0 and 2. A constant, −4, has been added to the values of E_{local}. The lines are drawn to guide the eye.

−6 ± 1 kcal/mol by Mitchell and Price[8] and later workers (see below).

Electrostatic Properties of Peptide Groups Near a Peptide End

The ESF and E_{local} results in Figures 3 and 4 indicate that the electrostatic properties of a peptide group depend on its proximity to a peptide end for residues close to one end (x = 2 or less), but not for more interior peptide groups. Ligand–peptide backbone interactions that occur in aqueous solution by an electrostatic mechanism may show similar behavior although the changes in ΔG values are likely to be smaller. An attractive case to investigate is the hydrogen exchange (HX) behavior of peptide groups in blocked alanine peptides, because some data are available and the ligands causing exchange are charged and are likely to interact with the peptide backbone by an electrostatic mechanism. In base-catalyzed exchange, the OH^- ion removes a proton from the peptide NH group; in acid-catalyzed exchange the H^+ ion adds to, and then exchanges places with, the proton on the peptide NH group. The basic or acidic pK of the peptide group determines the corresponding HX rate, provided that hydrogen exchange occurs by a diffusion-controlled reaction, as believed.[31,32]

The basic prediction made from the results shown here in Figures 3 and 4 is that HX rates are likely to vary with position for the first two or three peptide groups from either peptide end but not for interior peptide groups. Some data are available for short, blocked alanine ho-

mopeptides from Bai et al.[33] We quote values for $\Delta G^* = -RT\ln k_{HX}$, where k_{HX} is the rate of hydrogen exchange, and $\Delta\Delta G^*$ is $\Delta G^*(x,n) - \Delta G^*(x = 1, n = 1)$. The two peptide NH groups (x = 1,2) in the blocked alanine dipeptide (n = 1) have markedly different ΔG^* values[33] for base-catalyzed exchange ($\Delta\Delta G^* = 0.89$ kcal/mol) but similar values for acid-catalyzed exchange. For the central peptide NH proton (x = 2, n = 3) of the blocked A_3 tetrapeptide, both the base-catalyzed and acid-catalyzed exchange rates differ markedly from those of the blocked alanine dipeptide: $\Delta\Delta G^* = 0.62$ for base-catalyzed and -1.67 kcal/mol for acid-catalyzed exchange.[33] Thus, peptide groups close to the N-terminus do have position-dependent HX rates, as proposed; it is important to find out if the HX rates of interior NH groups in homopeptides are independent of position, as also proposed here.

Transfer free energy experiments on blocked glycine peptides typically show a strong peptide end effect.[34–36] In these experiments, the peptide typically is transferred from water to 1 M interacting ligand (e.g., osmolyte, denaturant, or sugar), the peptide solubility is measured both in water and in the ligand solution, and the transfer free energy is determined from the two solubilities. The subject has been clarified considerably by the recent finding of Auton and Bolen[36] that, if the peptide solubility is vanishingly small, peptide activity coefficients may be neglected and consistent transfer free energies are obtained from peptide concentrations measured on either the molar, molal, or mole fraction concentration scales. When the difference in transfer free energy is taken between two peptide studies in which the peptides differ by one glycyl residue ("constant increment method"[36]), the transfer free energy becomes independent of peptide length for blocked peptides with two or more glycyl residues. Auton and Bolen[36] omit transfer free energy data for blocked glycyl peptides with one or zero residues because their solubilities are not small enough to give transfer free energies that are independent of the concentration scale. The effect discussed here, namely a dependence of electrostatic properties on peptide length for peptides with two or fewer residues, may contribute to the peptide end effect they observe.

Group Additivity and the Neighboring Residue Effect

Neighboring residues in general affect the backbone conformation of a test residue, according to both experimental data[37,38] and structural data from the Protein Data Bank.[17,37] Moreover, this type of neighboring residue effect is expected from ESF calculations.[17] The existence of the neighboring residue effect means that some deviations from group additivity are generally present in peptide studies. Flory's hypothesis that the backbone conformation of a residue should be independent of its neighbors in denatured proteins (the "isolated pair" hypothesis[39]) has been highly influential in studies of the unfolded state of proteins. The neighboring residue effect means, however, that the isolated pair hypothesis is in general not valid. A different reason for the failure of Flory's isolated pair hypothesis, specific steric clashes, has been found in some specific cases.[40,41]

According to NMR studies of short peptides and also to structural data from the Protein Data Bank,[17,37] neighboring residues may be divided into two groups: the L group (aromatic and β-branched amino acids, FHITVWY) and the S group (all others, with Pro omitted; we also omit Gly). These two groups of neighboring residues produce either large effects (the L group) or small effects (the S group) on the backbone conformations (ϕ values) of *all* amino acids.[17,37] This result demonstrates that the backbone conformations of neighboring residues are not independent and that peptide electrostatic solvation, which depends on backbone conformation, is not independent of neighboring residues. The relation between the neighboring residue effect and ESF has been characterized for a specified range of ϕ-values in the polyproline II window ($-100° < \phi < -40°$; $90° < \psi < 180°$) of the "coil library" (residues not in helices and not in β-structure). The difference in $\langle \phi \rangle$ caused by having L versus S neighbors is on average 2.2°; similar values are found for almost all individual amino acids.[17]

Energetics of Peptide H-bonds in Folding

The Introduction states that ESF calculations provide a satisfactory framework for understanding both the enthalpy of solvation of amide polar groups (~ -12 kcal/mol, Fig. 2) and the enthalpy of forming the alanine peptide helix (-0.9 kcal/mol per residue).[10–12] Figure 2 shows that ΔH is nearly equal to ΔG^o for solvation of the polar groups of four different amides[23] and the ΔG^o values are fitted closely by the ESF values calculated with DelPhi.[18,23] Thus the relation between ESF and the enthalpy of amide solvation is straightforward.

To understand the ΔH of the alanine peptide helix, we first make the approximation (discussed above) that ESF $\sim$ ΔH for the interaction between water and the peptide group; this approximation applies both to the helix and to the unfolded peptide. Then we can write:

$$\Delta H(\text{helix/coil}) = \text{ESF(helix)} + \Delta H(\text{H-bond}) - \text{ESF(coil)} \quad (3)$$

The ESF of the H-bonded peptide group in an alanine helix is -2.5 kcal/mol at the center of a 15-residue helix.[23] For ΔH of the peptide H-bond, we use the gas phase energy computed by Ben-Tal et al.,[42] -6.6 kcal/mol. ESF(coil) depends on backbone conformation[7,43] and depends also on the extent to which an unfolded peptide bends back on itself.[23] Consequently, we solve Equation (3) for ESF(coil) and ask if a reasonable value is obtained: ESF(coil) = $+0.9 - 2.5 - 6.6 = -8.2$ kcal/mol. This value may be compared with the ESF of interior alanine peptide groups in the polyproline II conformation, -9.1 kcal/mol, or in the extended-β conformation, -8.5 kcal/mol.[17] Because bending back of the unfolded peptide on itself reduces the access of peptide groups to water and thereby reduces their negative ESF values,[23] the agreement is satisfactory. Even without allowing for bending back, the agreement is

within ±1 kcal/mol, which is set by the approximation $\Delta H \sim ESF$ used here (see Fig. 2).

There has been a longstanding discussion about whether peptide H-bonds do or do not stabilize protein folding.[43–45] When the question is limited to the ΔH values rather than the ΔG^o values for peptide H-bonds, the answer given here is as follows. The ΔH for the alanine peptide helix is known to be -0.9 ± 0.1 kcal/mol[10–12] and, as discussed above, solvent-exposed peptide H-bonds stabilize this helix. The terms contributing to ΔH(helix/coil) (see Eq. [3]) include the term ESF(helix) = −2.5 kcal/mol, which will drop to 0 when the helix is buried via folding.[46] Consequently, although the peptide H-bonds in a solvent-exposed alanine helix stabilize the helix by −0.9 kcal/mol, burying the helix through protein folding should cause the H-bonded peptide group to be destabilizing by $(2.5 - 0.9) = 1.6$ kcal/mol.

According to the analysis in Equation (3), solvent-exposed peptide H-bonds should provide an important source of stability for molten globule folding intermediates.[23] Because side chains shield neighboring peptide groups from water and reduce −ESF values,[17] the peptide H-bonds which have other neighboring side chains other than alanine should contribute less to helix stability. Richardson et al.[12] find that the ΔH of peptide helix formation does vary substantially for several types of nonpolar (and also polar) residues and −ΔH for helix formation is larger for alanine than for isoleucine and valine.

CONCLUSIONS

The electrostatic character of polar solvation means that the principle of group additivity must be used with great caution when applied to peptide groups. Group additivity requires that the groups under study are independent of each other. There are two main factors that tend to prevent the solvation thermodynamics of neighboring peptide groups from being independent. First, polar or electrostatic solvation of the peptide group varies with the peptide dipole–dipole interactions made by adjacent peptide groups.[15,28] These dipole–dipole interactions depend strongly on geometry and therefore on backbone conformation. Secondly, polar solvation is affected also by neighboring side chains that restrict the access of water to peptide groups. Thus, in general neighboring peptide groups do not have independent polar or electrostatic solvation and use of the principle of group additivity must be examined carefully. Hydrogen exchange data are used here to show here that interactions between a polar ligand and the peptide backbone are also likely to be position dependent if the ligand interacts with the backbone by an electrostatic mechanism.

ACKNOWLEDGMENTS

The authors would like to thank Wayne Bolen for discussion. This work was supported by a grant to FA from the Ministry of Education and Science of Slovenia.

REFERENCES

1. Dill KA. Additivity principles in biochemistry. J Biol Chem 1997; 272:701–704.
2. Makhatadze GI, Privalov PL. Contribution of hydration to protein folding thermodynamics. I. The enthalpy of hydration. J Mol Biol 1993;232:639–659.
3. Klotz IM, Franzen JS. Hydrogen bonds between model peptide groups in solution. J Am Chem Soc 1962;84:3461–3466.
4. Klotz IM, Farnham SB. Stability of an amide hydrogen bond in an apolar environment. Biochemistry 1968;7:3879–3882.
5. Roseman MA. Hydrophilicity of polar amino acid side-chains is markedly reduced by flanking peptide bonds. J Mol Biol 1988;200: 513–522.
6. Roseman MA. Hydrophobicity of the peptide C═O· · ·H-N hydrogen-bonded group. J Mol Biol 1988; 201:621–623.
7. Baldwin RL. In search of the energetic role of peptide hydrogen bonds. J Biol Chem 2003;278:17581–17588.
8. Mitchell JBO, Price SL. On the relative strengths of amide· · ·amide and amide· · ·water hydrogen bonds. Chem Phys Lett 1991;180: 517–523.
9. Marqusee S, Robbins VH, Baldwin, RL. Unusually stable helix formation in short alanine-based peptides. Proc Natl Acad Sci USA 1989;86:5286–5290.
10. Lopez MM, Chin DH, Baldwin RL, Makhatadze GI. The enthalpy of the alanine peptide helix measured by isothermal titration calorimetry using metal binding to induce helix formation. Proc Natl Acad Sci USA 2002;99:1298–1302.
11. Goch G, Maciejczyk M, Oleszczuk M, Stachowiak D, Malicka J, Bierzynski A. Experimental investigation of initial steps of helix propagation in model peptides. Biochemistry 2003;42;6840–6847.
12. Richardson JM, Lopez MM, Makhatadze GI. Enthalpy of helix-coil transition: missing link in rationalizing the thermodynamics of helix-forming propensities of the amino acids. Proc Natl Acad Sci USA 2005;102:1413–1418.
13. Fersht AR. The hydrogen bond in molecular recognition. Trends Biochem Sci 1987;12:301–304.
14. Smith JD, Cappa CD, Wilson KR, Messer BM, Cohen RC, Saykally RJ. Energetics of hydrogen bond network rearrangement in liquid water. Science 2004;306:851–853.
15. Avbelj F. Amino acid conformational preferences and solvation of polar backbone atoms in peptides and proteins. J Mol Biol 2000;300:1335–1359.
16. Avbelj F, Baldwin RL. Role of backbone solvation and electrostatics in generating preferred peptide backbone conformations: distributions of phi. Proc Natl Acad Sci USA 2003;100:5742–5747.
17. Avbelj F, Baldwin RL. Origin of the neighboring residue effect on peptide backbone conformation. Proc Natl Acad Sci USA 2004;101: 10967–10972.
18. Sitkoff D, Sharp KA, Honig B. Accurate calculation of hydration free energies using macroscopic solvent models. J Phys Chem 1994;98:1978–1988.
19. Born M. The volume and heat of hydration of ions. Z. Phys. 1920;1:45–48.
20. Rashin A, Honig B. Reevaluation of the Born model of ion hydration. J Phys Chem 1985;89:5588–5593.
21. Noyes RM. Thermodynamics of ion hydration as a measure of effective dielectric properties of water. J Am Chem Soc 1962;84: 513–522.
22. Robinson RA, Stokes RH. Electrolyte solutions. London: Butterworths; 1955.
23. Avbelj F, Luo P, Baldwin RL. Energetics of the interaction between water and the helical peptide group and its role in determining helix propensities. Proc Natl Acad Sci USA 2000;97: 10786–10791.
24. Wolfenden R. Interaction of the peptide bond with solvent water: a vapor phase analysis. Biochemistry 1978;17:201–204.
25. Della Gatta G, Barone G, Elia V. Enthalpies of solvation for N-alkylamides in water and in carbon tetrachloride at 25°C. J Solution Chem 1986;15:157–167.
26. Dunitz JD. The entropic cost of bound water in crystals and biomacromolecules. Science 1994;264:670.
27. Florian J, Warshel A. Langevin dipoles model for ab initio calculations of chemical processes in solution: parameterization and application to hydration free energies of neutral and ionic solutes and conformational analysis in aqueous solution. J Phys Chem B 1997;101:5583–5595.
28. Avbelj F, Moult J. Role of electrostatic screening in determining protein main chain conformational preferences. Biochemistry 1995;34:755–764.

29. Brant DA, Flory PJ. The role of dipole interactions in determining polypeptide configurations. J Am Chem Soc 1965;87:663–664.
30. Wada A. The α-helix as an electric macrodipole. Adv Biophys 1976;9:1–63.
31. Englander SW, Kallenbach, NR. Hydrogen exchange and structural dynamics of proteins and nucleic acids. Quart Rev Biophys 1984;16:521–655.
32. Fogolari F, Esposito G, Viglino P, Briggs JM, McCammon JA. pKa shift effects on backbone amide base-catalyzed hydrogen exchange rates in peptides. J Am Chem Soc 1998;120:3735–3738.
33. Bai Y, Milne JS, Mayne L, Englander SW. Primary structure effects on peptide group hydrogen exchange. Proteins 1993;17:75–86.
34. Cohn EJ, Edsall JT. Proteins, amino acids and peptides as ions and dipolar ions. New York: Reinhold Publishing Company; 1943.
35. Nandi PK, Robinson DR. Effects of urea and guanidine hydrochloride on peptide and nonpolar groups. Biochemistry 1984;23:6661–6668.
36. Auton M, Bolen DW. Additive transfer free energies of the peptide backbone unit that are independent of the model compound and the choice of concentration scale. Biochemistry 2004;43:1329–1342.
37. Penkett CJ, Redfield C, Dodd I, Hubbard J, McBay DL, Mossakowska DE, Smith RAG, Dobson CM, Smith LJ. NMR analysis of main-chain conformational preferences in an unfolded fibronectin-binding protein. J Mol Biol 1997;274:152–159.
38. Chen K, Liu Z, Zhou C, Shi Z, Kallenbach NR. Neighbor effect on PPII conformation in alanine peptides. J Am Chem Soc 2005;127:10146–10147.
39. Flory PJ. Statistical mechanics of chain molecules. New York: Interscience. 1969.
40. Pappu R, Srinivasan R, Rose GD. The Flory isolated-pair hypothesis is not valid for polypeptide chains: implications for protein folding. Proc Natl Acad Sci USA 2000;97:12565–12570.
41. Fitzkee NC, Rose GD. Steric restrictions in protein folding: an α-helix cannot be followed by a contiguous β-strand. Protein Sci 2004;13:633–639.
42. Ben-Tal N, Sitkoff D, Topol IA, Yang AS, Burt SK, Honig B. Free energy of amide hydrogen bond formation in vaccuum, in water, and in liquid alkane solution. J Phys Chem B 1997;101:450–457.
43. Honig B, Yang AS. Free energy balance in protein folding. Adv Protein Chem 1995;46:27–58.
44. Myers JK, Pace CN. Hydrogen bonding stabilizes globular proteins. Biophys J 1996;71:2033–2039.
45. Pace CN. Polar group burial contributes more to protein stability than nonpolar group burial. Biochemistry 2001;40:310–313.
46. Avbelj F, Baldwin RL. Role of backbone solvation in determining thermodynamic β propensities of the amino acids. Proc Natl Acad Sci USA 2002;99:1309–1313.