Solvation and the Energetics of Protein Folding

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I. Background

1. Proteins are synthesized as chains of amino acid residues joined in peptide (NHCO) linkage, ...-CONH-HCR_1-CONH-HCR_2-...

1.a, There are 20 different amino acids (20 R groups).

1.b, The newly synthesized protein is unfolded (U). A protein does not become biologically active until it folds into a specific 3D structure. The product of folding (U→N) is the native protein (N).

1.c, Nonpolar side chains (e.g., R = -CH_2-CH-$(CH_3)_2$, leucine) become buried in the protein interior via folding.

1.d, Peptide groups make peptide H-bonds (-NH•••OC-) linking adjacent chain segments.

2. The driving force for specific folding is the change in free energy (ΔG) of the protein molecule.

2.a, Solvation (interaction with water), both of nonpolar R groups and polar peptide groups, provides the major part of the free energy change on folding.
2.b, Solvation of nonpolar R groups is energetically unfavorable, hence nonpolar R groups become buried on folding.
2.c, Solvation of polar peptide groups is energetically favorable in the unfolded protein, but is balanced energetically (but only approximately) by formation of peptide H-bonds in the folded protein. ~70% of peptide groups make peptide H-bonds (-NH•••OC-) in N.
2.d, For many years, protein chemists considered that only burial of nonpolar R groups is energetically important in folding.
2.e, Today they begin to consider also the energetics of forming peptide H-bonds.
3. Solvation energetics is a critical subject in modern structure prediction work!

II. Energetics of Side Chain Burial

A. Kauzmann's model (1959): treat protein interior as an organic liquid

1. Use model compounds to represent protein side chains, e.g., toluene for the phenylalanine side chain.
2. Use liquid-liquid transfer data to find the energetics of burying nonpolar side chains, e.g, take the transfer of toluene from liquid toluene→water and take $\Delta G^{o} = 5.2$ kcal/mol, calculated from solubility of toluene in water.
2.a, The net free energy change for folding a small (~100 residues) protein is only ~-10 kcal/mol and this is equivalent to burying only two phenylalanine side chains, according to Kauzmann's model.

B. Packing-Desolvation Model: treat protein interior as an organic crystal (Privalov & Gill, 1988)

1. Rationale: the protein interior is close-packed like an organic crystal and its packing does not resemble an organic liquid, as assumed in Kauzmann's model.
2. To desolvate nonpolar side chains, use water→gas phase transfer data: i.e., measure the equilibrium ΔG and ΔH for transfer of a

hydrocarbon solute between the vapor phase and aqueous solution.

3. To compute the packing energy of nonpolar side chains in N , use the Lennard-Jones pairwise potential and the protein structural coordinates.

$$E = \varepsilon[(R_{min}/R)^{12} - 2\,(R_{min}/R)^{6}]$$

3.a, Program developed and tested by Lazaridis, Archontis & Karplus (LAK), 1995. To validate the program, LAK compare vdW energies for liquid and crystalline alkanes with vdW energies computed from protein R values.

3.b, Results from LAK for the binding energy of the CH_3 group (based on energy-minimized structures) are:

alkane crystals, –3.8 to - 4.3 kcal/mol
liquid alkanes, -1.7 to - 2.1 kcal/mol
myoglobin, -3.6±0.8 kcal/mol

Note the large (2-fold) energetic difference between the alkane liquid and crystal, and the protein value is close to the crystal.

III. Unfolding Enthalpy in Vacuum: Solvation Compared with H-Bonds and vdW Interactions

1. Cycle:

N → U (vacuum)
↓ ↑
N → U (water)

1.a, In 1995 Lazaridis, Archontis & Karplus (LAK) simulated the enthalpy of N → U (vacuum) by using CHARMM to estimate the ΔH of breaking HB and vdW interactions.

1.b, Makhatadze & Privalov (MP) estimated ΔH for N → U (vacuum) by summing data for the other 3 sides of the cycle:
(1) solvate N
(2) unfold N to U in water
(3) desolvate U

2. They give the following results for hen lysozyme (129 residues).

2.a, LAK: vdW, 738 kcal/mol
HB, 351 kcal/mol
"other", 27 kcal/mol
total, 1116 kcal/mol

2.b, MP: polar solvation, 1769 kcal/mol
nonpolar solvation, 261 kcal/mol
ΔH, N → U (water), 58 kcal/mol
total, 2088 kcal/mol

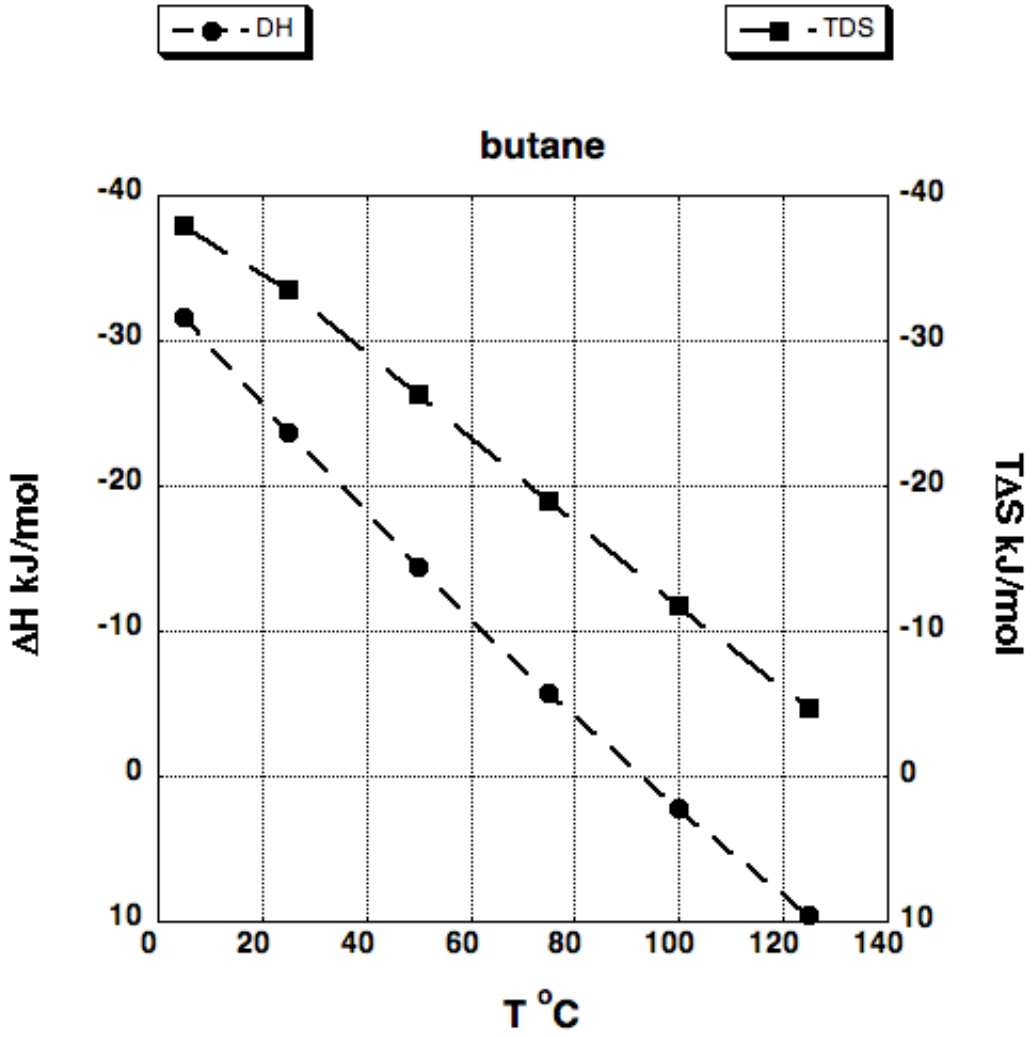
2.c, There is a ~ 2-fold discrepancy between values for the ΔH of N → U (vacuum) (LAK, 1116 vs. 2088, MP).
3. The discrepancy is probably caused chiefly by the ΔH (1769 kcal/mol) that MP assigned to desolvating peptide groups. A quite different value can be assigned as follows.
3.a, The number of fully H-bonded peptide groups in N, with at least one HB made to both the NH and CO groups, (termed the "reduced HB" by Stickle, Presta, Dill & Rose, 1992) scales as 0.714 times the number of residues. For hen lysozyme, this number is (0.714)(129) = 92.
3.b, Study of the ΔH for desolvating a solvent-exposed peptide group gives ~ 8.5±1.5 kcal/mol (see below). Then (92)(8.5±1.5) = 782±138 kcal/mol as the value for desolvating peptide groups that are buried in N but solvent-exposed in U. Substituting this value for the MP estimate of 1769 kcal/mol gives a revised MP total of 1101±138 kcal/mol, close to the LAK total of 1116 kcal/mol.
3.c, Note that the measured ΔH for N → U in water is small (58 kcal/mol) compared to

unfolding in vacuum (1101 kcal/mol) because the unfavorable ΔH of breaking HB and vdW interactions nearly cancels the favorable ΔH of solvating polar and nonpolar groups.

IV. Solvation Thermodynamics of Hydrocarbons

1. Hydrocarbon solvation (defined as transfer of a hydrocarbon solute from the gas phase to water) has an unfavorable ΔG at all temperatures although ΔH is favorable except at high temperatures. The reason is that the energetics of hydrocarbon solvation by water are complex and very different from the behavior of "regular solutions".
2. ΔH and ΔS both approach 0 at temperatures near 100° C (for ΔH) or well above 100° C (for ΔS). Above the temperature at which ΔH = 0, ΔG becomes enthalpy-driven and reaches its

maximum value when $\Delta S = 0$.



V. Solvation Thermodynamics of Peptide Groups

1. Solvation energetics of the peptide group are in a state of flux. Calorimetrists have assumed that the principle of group addivity is applicable to the interaction between water and the polar peptide group, but current work indicates this assumption is not valid (Avbelj &Baldwin,

2006; Della Gatta et al., 2006; Avbelj & Baldwin, 2008).

2. To understand this situation better, it is helpful to review the solvation energetics of monovalent ions, which are well understood (Born, 1920; Latimer et al., 1939).

2.a, The solvation free energies can be fitted by the Born equation (Born, 1920) for the charging of a sphere in vacuum followed by its transfer to a solvent of high dielectric constant (water, D =78); q is the charge and r is the radius of the ion, while N is Avogadro's number.

$$-\Delta G = N(q^2/2r)[1-(1/D)]$$

2.b, The ΔH values are huge (~100 kcal/mol), while ΔH is close in value and almost proportional to ΔG.

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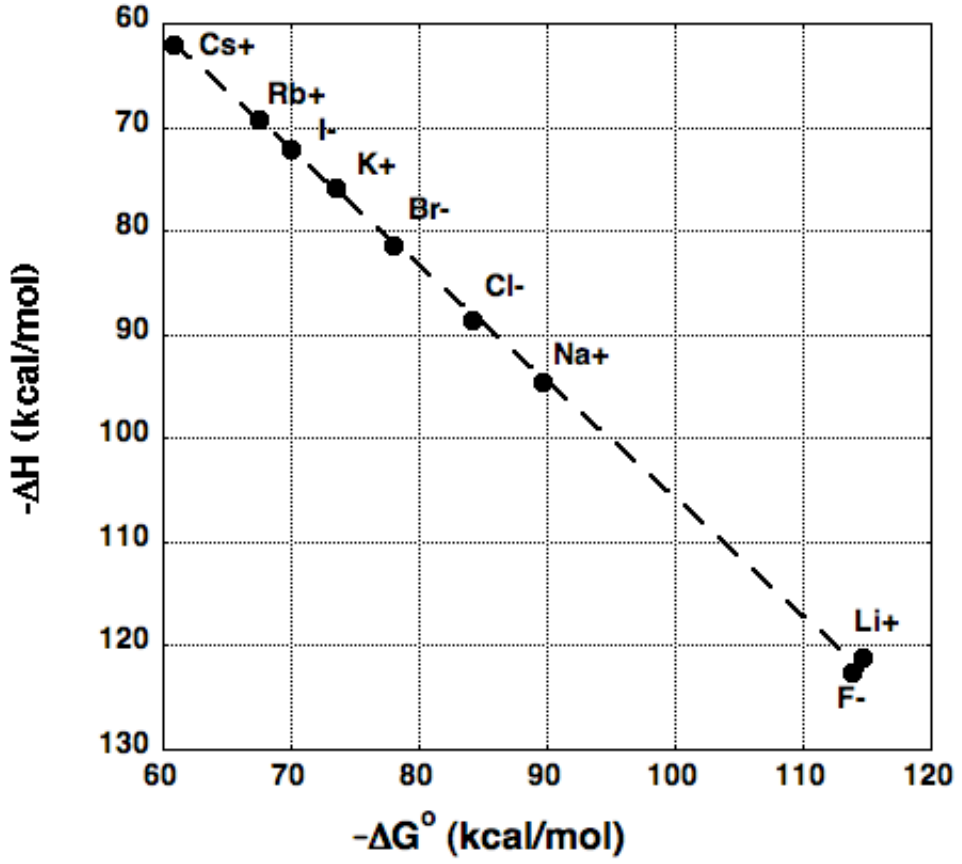
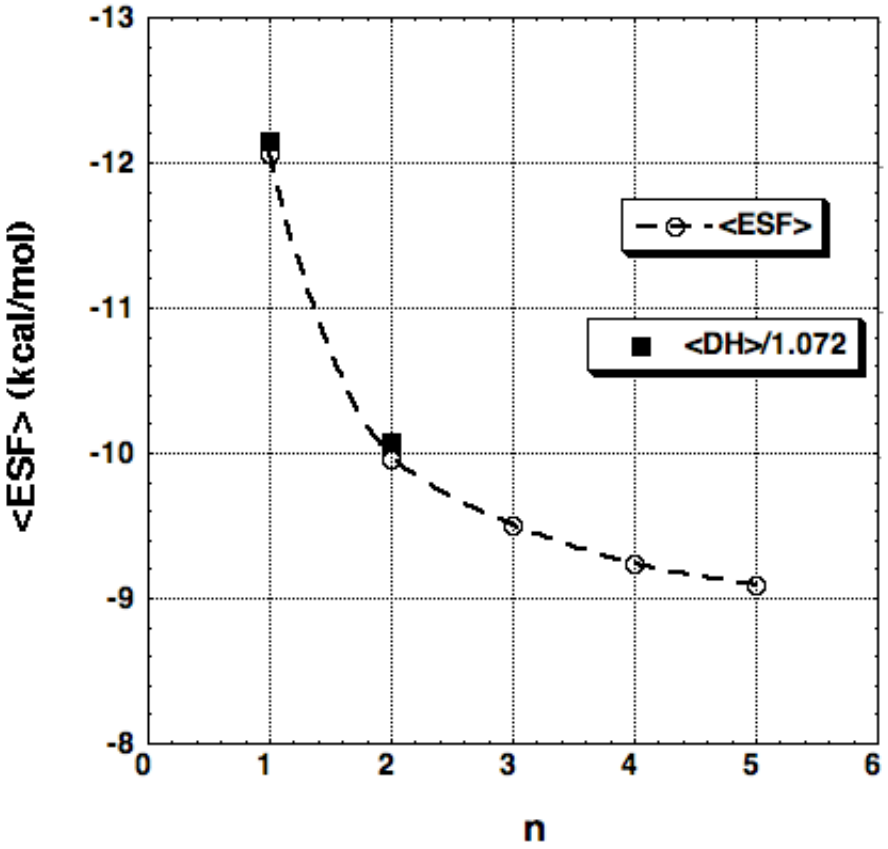


3. The atoms of the peptide group (NHCO) all carry large partial charges (~0.5e) and consequently solvation of the strongly polar NHCO group may be estimated by algorithms such as DelPhi (Honig and coworkers) or UHBD (McCammon and coworkers) that employ numerical solution of the approximate Poisson-Boltzmann equation. ΔG values found in this

way are referred to here as esf (electrostatic solvation free energy) values.

4. Calculations of esf for alanine peptides of varying chain lengths show that esf of a given NHCO group depends strongly on the presence or absence of adjoining NHCO groups. This effect implies that the ΔH for solvation of a peptide group likewise depends strongly on neighboring peptide groups. This effect is confirmed by calorimetric data for dipeptide

analogs obtained by Della Gatta et al. (2006).



5. A similar effect is seen in the hydrogen exchange (HX) rates of short alanine or glycine peptides: there is a ~100-fold increase in the HX rate of the NH group of a mono-amide when adjoining NHCO groups are present on either side (Molday et al., 1972). The base-catalyzed HX rate depends on the the alkaline pK_a of the NH group, The shift in pK_a caused by the

presence of adjoining NHCO groups may be calculated by the UHBD algorithm (Fogolari et al., 1998).

VI. Conclusions

1. In the energetics of protein folding, solvation of the polar peptide groups is at least equally important as the solvation of the nonpolar side chains.
2. Solvation of nonpolar side chains has been analyzed in the past by Kauzmann's model that treats the protein interior as an organic liquid. Current work focuses on the packing-desolvation model that treats the protein interior as a semi-crystalline solid.
3. The breakdown of group additivity for solvation of peptide groups appears to be the major reason for the ~2-fold discrepancy found in 1995 between protein folding enthalpies in vacuum simulated by Lazaridis, Archontis and Karplus, using CHARMM, versus values estimated by Makhatadze and Privalov, using calorimetry.
4. (Not discussed here for lack of time.) An assumed proportionality between ΔG for

solvation of nonpolar side chains and ASA (accessible surface area) is widely used today but is contradicted by experimental data from Simonson & Brünger, 1994.

VII. References

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