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REVIEW

Side-chain conformational entropy in protein folding

ANDREW J. DOIG[1] AND MICHAEL J.E. STERNBERG[2]

[1] Department of Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science and Technology, Manchester M60 1QD, United Kingdom
[2] Biomolecular Modelling Laboratory, Imperial Cancer Research Fund, London WC2A 3PX, United Kingdom

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Abstract

An important, but often neglected, contribution to the thermodynamics of protein folding is the loss of entropy that results from restricting the number of accessible side-chain conformers in the native structure. Conformational entropy changes can be found by comparing the number of accessible rotamers in the unfolded and folded states, or by estimating fusion entropies. Comparison of several sets of results using different techniques shows that the mean conformational free energy change ($T\Delta S$) is 1 kcal·mol^{-1} per side chain or 0.5 kcal·mol^{-1} per bond. Changes in vibrational entropy appear to be negligible compared to the entropy change resulting from the loss of accessible rotamers. Side-chain entropies can help rationalize α-helix propensities, predict protein/inhibitor complex structures, and account for the distribution of side chains on the protein surface or interior.

Keywords: conformational entropy; internal rotation; protein folding; protein stability; side chain

The major force opposing protein folding is loss of conformational entropy. A dynamic unfolded protein is able to access a vast number of conformations. In particular, its side chains are sampling many different rotameric states. When a protein folds, side chains that are buried in the close-packed protein core are generally restricted to a single conformation. Even side chains that remain exposed to solvent on the protein surface can be more restricted than in the unfolded state. Entropy is related directly to the number of different conformations a particular state can adopt. This reduction in the number of side-chain rotamers that are populated results in a sizeable loss of entropy opposing protein folding.

Side-chain conformational entropy is, of course, only one of the many aspects of the thermodynamics of protein stability that are generally considered to be primarily entropic rather than enthalpic. Probably the best recognized and most studied of these is the hydrophobic effect that primarily results from the freeing up of ordered waters when nonpolar groups are removed from solvent. Many workers have established scales for residue hydrophobicity. Indeed Cornette et al. (1987) cited 46 different scales.

Another well-studied effect is disulfide bridge formation. Many workers attribute the restriction in conformational freedom of the unfolded state in the crosslinked chain compared to the uncrosslinked molecule as the main reason for the increase in protein stability resulting from a disulfide bridge. Recently, for example, Harrison and Sternberg (1994) showed that entropic restriction in the unfolded state provides a good model for observed disulfide connectivity patterns and loop lengths in short sequences (<75 residues). However, Doig and Williams (1991) proposed in addition that disulfides decrease the surface area of the unfolded state. This reduces the size of the hydrophobic effect and causes enthalpic stabilization and entropic destabilization of the folded state when a disulfide is present. Experimental evidence that could distinguish these two models remains inconclusive (Betz, 1993).

In contrast to these effects, the conformational and vibrational entropy of both main chain and side chain have been far less well characterized. Here we are interested in the change in entropy occurring during folding. When a protein folds, entropic effects from changing its configuration can arise from two sources: First, there will be a change in the number of conformations (essentially rotamers) populated (ΔS_{conf}). Figure 1 shows a change in ΔS_{conf} as a rotamer is restricted from three conformations to one. Second, the width of an allowed potential en-

Reprint requests to: Andrew J. Doig, Department of Biochemistry and Applied Molecular Biology, University of Manchester Institute of Science and Technology, P.O. Box 88, Manchester M60 1QD, UK; e-mail: andrew.doig@umist.ac.uk.

Abbreviations: ΔS_{conf}, change in entropy from decreasing the number of energetically accessible rotamers; ΔS_{vib}, loss of vibrational entropy; ΔS_{config}, total entropy change from restricting side-chain motion (sum of ΔS_{conf} and ΔS_{vib}); W, number of different conformations adopted in the unfolded state; σ, symmetry number for rotation; p_i, fractional population of a rotamer; ΔS_{fus}, fusion (liquid → solid) entropy change.

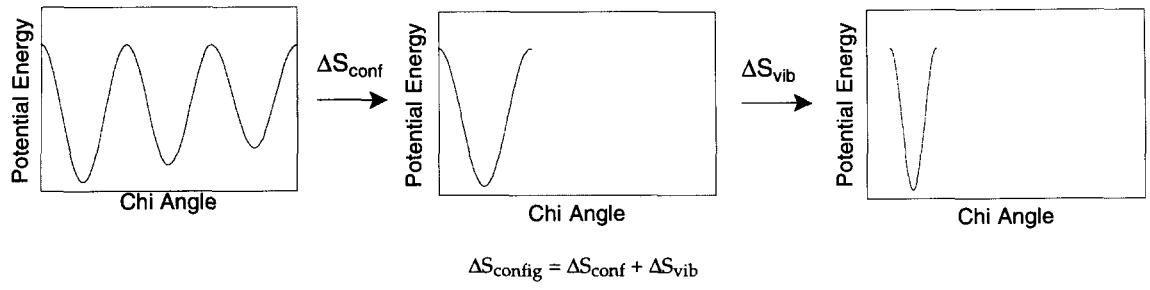

Fig. 1. Changes in the configurational entropy (ΔS_{config}) of a side chain arise from two sources: a reduction in the number of rotamers populated (ΔS_{conf}) and the restriction of torsional vibrations about an energy minimum (ΔS_{vib}). Outside the accessible range of χ angles, the energy is very high and unknown.

ergy well might narrow in a close-packed folded protein. This corresponds to the bond being restricted to a smaller range of dihedral angles and results in a loss of vibrational entropy (ΔS_{vib}; Fig. 1). This is a torsional vibration about a covalent bond axis. The sum of both these effects is configurational entropy ($\Delta S_{config} = \Delta S_{conf} + \Delta S_{vib}$).

Karplus et al. (1987) used molecular dynamics simulations to estimate vibrational and conformational entropy during folding. They suggested that, although the magnitude of absolute vibrational entropy is nearly an order of magnitude larger than conformational entropy, the most important change on folding is in conformational entropy. Although attempts to dissect the relative magnitudes of different thermodynamic effects have been criticized as not formally correct (Mark & van Gunsteren, 1994), it remains helpful in understanding protein stability to estimate these individual contributions (Boresch et al., 1994). In this article, we review the renewed interest in estimating side-chain conformational entropy. Rotatable side-chain χ angles that can be restricted in protein folding are numbered according to convention (IUPAC-IUB Commission on Biochemical Nomenclature, 1969, 1970).

Changes in side-chain conformational entropy on folding (hereafter abbreviated ΔS_{conf}) were first discussed nearly 30 years ago (Némethy et al., 1966). In recent years, there has been much renewed interest in this area. In the last five years, a number of papers have been published that make estimates of ΔS_{conf} by independent methods. Here we discuss each of these methods and compare their results. This allows us to draw some general conclusions on the size and importance of ΔS_{conf}.

Side-chain conformational entropy from the Boltzmann equation

The most straightforward method uses the Boltzmann equation (Equation 1) directly to calculate entropy:

$$\Delta S_{conf} = -R \ln W; \quad (1)$$

W is the number of different conformations adopted in the unfolded state. This can be taken to be 3 for each sp^3-sp^3 single bond (Novotny et al., 1989; Krystek et al., 1993) if it is assumed that each rotamer is populated equally in the unfolded state (i.e., each bond is 33% *gauche*$^+$, 33% *gauche*$^-$, and 33% *trans*) and that the conformational entropy of the folded state is zero (i.e., 100% in a single rotamer). The simplest method is therefore to estimate ΔS_{conf} as $-R \ln 3$ (-2.2 cal·K^{-1} mol^{-1}) per rotatable bond (Novotny et al., 1989).

The symmetry about the bond axis (σ) must also be considered. For example, the bond to the aromatic ring in Phe ($\chi 2$) has twofold symmetry, because an identical state is reached if this bond is rotated by 180°. The entropy in the folded state is thus $R \ln 2$, instead of zero ($R \ln 1$), and ΔS_{conf} for this bond is therefore smaller. Similarly, $\chi 2$ of Tyr, $\chi 2$ of Asp, and $\chi 3$ of Glu also have twofold symmetry and hence a reduced value of ΔS_{conf}.

A more sophisticated approach is to take into account that the rotamers in the unfolded state are not equally populated. Figure 1 shows three conformations accessible in the unfolded state with different energies. A state with higher energy will be populated less often. Némethy et al. (1966) and Finkelstein and Janin (1989) used Equation 1, with W as 2-3 for each rotatable bond. A more accurate approach is to use Equation 2, where p_i is the fractional population of each rotamer state i in the unfolded state.

$$\Delta S_{conf} = -R \sum_i p_i \ln p_i \quad (2)$$

The populations of each rotamer in the unfolded state cannot yet be observed directly. Instead, Pickett and Sternberg (1993) assumed that the conformations adopted by side chains in protein crystal structures are representative of unfolded conformations. The distribution of side-chain rotamers at interior positions of α-helices is unusual, however, so they were excluded. The results of their survey were used to determine ΔS_{conf} using Equation 2. It was necessary to correct some residues for symmetry (discussed above) and they added a term for groups that showed essentially free rotation in the unfolded state, restricted by hydrogen bonding when buried. Their results are given in Table 1.

Abagyan and Totrov (1994) used a similar approach, finding p_i for all $\chi 1$ and $\chi 2$ angles by surveying 161 dissimilar protein domains. Additional terms were added for $\chi 3$ and $\chi 4$ rotations and symmetry corrections were made. Their results are given in Table 1.

Koehl and Delarue (1994) found p_i for each rotatable bond in the folded state by calculating the energy of each rotamer. The energies were calculated for different conformations of nearby rotamers and weighted by how often each neighboring conformation was adopted. The entropy of the unfolded state was found using Equation 1, where W is the number of possible

Table 1. *Changes in side-chain conformational entropy ($T\Delta S$) on protein folding at 300 K (kcal·mol^{-1})*

Residue	Number χ angles	$T\Delta S_{conf}$[a] Pickett and Sternberg	Abagyan and Totrov	Koehl and Delarue	Blaber et al.	Creamer and Rose	Lee et al.	Mean	$T\Delta S_{fus}$[b]	Hydrophobicity[c]	Absolute TS[d]
Ala	0	0	0	0	0	0	0	0	0	−0.42	0
Arg	4	−2.03	−2.13	−1.21			−2.13	−1.88		1.37	−13.7
Asn	2	−1.57	−0.81	−0.75			−0.99	−1.03	−1.24	0.82	−5.6
Asp	2	−1.25	−0.61	−0.65			−0.60	−0.78	−1.37	1.05	−4.2
Cys	2	−0.55	−1.14	−0.63			−1.06	−0.85	−0.65	−1.34	−3.9
Gln	3	−2.11	−2.02	−1.29			−1.51	−1.73	−1.91	0.30	−7.8
Glu	3	−1.81	−1.65	−1.31			−1.06	−1.46	−2.04	0.87	−6.4
Gly	0	0	0	0			0	0		0	0
His	2	−0.96	−0.99	−0.92	−0.89		−1.00	−0.95	−1.64	−0.18	−4.5
Ile	2	−0.89	−0.75	−0.94	−0.79	−0.67	−0.52	−0.76	−0.83	−2.46	−2.6
Leu	2	−0.78	−0.75	−0.94	−0.69	−0.58	−0.49	−0.71	−0.83	−2.32	−2.7
Lys	4	−1.94	−2.21	−1.63			−1.76	−1.89	−3.50	1.35	−9.3
Met	3	−1.61	−1.53	−1.24		−1.53	−1.37	−1.46	−1.54	−1.68	−4.4
Phe	2	−0.58	−0.58	−0.65	−0.61	−0.87[e]	−0.42	−0.62	−0.93	−2.44	−3.1
Pro	0	0	0	−0.30			0	−0.06		−0.98	0
Ser	2	−1.71	−1.19	−0.43			−1.10	−1.11	−0.89	0.05	−3.9
Thr	2	−1.63	−1.12	−0.57			−0.99	−1.08	−0.73	−0.35	−3.9
Trp	2	−0.97	−0.97	−1.14	−0.88	−1.16	−0.82	−0.99	−1.88	−3.07	−4.4
Tyr	3	−0.98	−0.99	−1.07		−1.76[e]	−0.83	−1.13	−1.35	−1.31	−5.6
Val	1	−0.51	−0.50	−0.62	−0.46	−0.42	−0.04	−0.43	−0.15	−1.66	−1.3
Total	41	−21.88	−19.94	−16.29				−18.92			−87.3
Mean	2.05	−1.09	−1.00	−0.81				−0.95			−4.37

[a] References: Pickett and Sternberg (1993); Abagyan and Totrov (1994); Koehl and Delarue (1994); Blaber et al. (1994); Creamer and Rose (1994); Lee et al. (1994).
[b] Sternberg and Chickos (1994).
[c] Fauchère and Pliska (1983).
[d] Doig et al. (1993).
[e] Corrected for symmetry.

rotamers for each side chain in the rotamer library of Tuffery et al. (1991). The difference between these quantities gives ΔS_{conf} (Table 1).

Creamer and Rose (1994) used an acetyl-(Ala)$_5$-X-(Ala)$_5$-NMe peptide in a nonhelical state as a model for an unfolded protein and found the rotamer populations of residue X by Monte Carlo simulations for eight residues. Blaber et al. (1994) surveyed the populations of $\chi 1$ and $\chi 2$ in nonhelical structure for seven residues.

Table 1 gives these results at 300 K and the mean values of $T\Delta S_{conf}$ for each residue. Each of these can be compared to the hydrophobicity of the side chains (Fauchère & Pliska, 1983), given relative to Gly. It is seen that side-chain conformational entropy is close to hydrophobicity in magnitude and hence importance in protein stability. The mean value of $T\Delta S_{conf}$ per residue is −0.95 kcal·mol^{-1}; the mean value of $T\Delta S_{conf}$ per rotatable χ angle is −0.46 kcal·mol^{-1}. The agreement between the results for various groups is best for nonpolar residues, because the treatment of side chains involved in hydrogen bonds varied. Some variation also arises from assigning different number of allowed rotamers to each χ angle and in the treatment of the folded state. Each of these scales correlates well with any other; the least-squares correlation coefficients when each scale is plotted against each other vary from 0.73 to 0.99 (not shown).

Absolute entropies

As mentioned above, each of these methods has assumed that the potential energy well for each rotamer has same width in the unfolded and folded states (i.e., $\Delta S_{vib} = 0$; Fig. 1). It is possible, however, that the well is narrower in the folded state, restricting the bond to a smaller range of dihedral angles and hence giving larger ΔS_{conf} (i.e., $\Delta S_{vib} < 0$). At the opposite extreme, we can consider the thermodynamic changes when motion stops completely and a bond is fixed at the base of one well. This gives the maximum possible entropy change for restricting side chains and is the absolute entropy (i.e., entropy change upon cooling to absolute zero). These maximum possible entropy changes have been calculated by Doig et al. (1993) (Table 1). ΔS_{config} is ≈4 times larger than ΔS_{conf} by this method, but the different sets of results correlate well. (The correlation coefficients when the absolute entropy is plotted against each of the scales of ΔS_{conf} varies from 0.76 to 0.95.) This suggests that only ≈25% of the absolute entropy of a side chain is lost when a protein folds. The remainder is retained in the form of a torsional vibration. S_{vib} calculated by Karplus et al. (1987) is larger than the S_{vib} of Doig et al. (1993) because the former work considered degrees of freedom in addition to just dihedral angle rotation, such as bond angle variation and larger scale motions.

Lee et al. (1994) calculated side-chain entropies for residue X in a helix $(Ala)_4$-X-$(Ala)_4$ by three methods. First, they found S_{conf} using the Equation 2, determining p_i by calculating the energy as each dihedral angle is varied (Table 1). Second, they calculated S_{vib} by two methods to find the total S_{config}. S_{vib} was found by assuming that the torsional vibration is a simple harmonic oscillator, or by integrating the Hamiltonian for the vibration with terms for kinetic and potential energies. The sum of S_{conf} and S_{vib} gives S_{config} for a side chain on the surface of an α-helix. They concluded that the frequency of each torsional oscillation does not change when an internal rotation is restricted (i.e., $\Delta S_{vib} = 0$), justifying using changes in conformational entropy as the total change in configurational entropy.

Entropies of fusion

The core of a protein is as close packed as an organic crystal (Richards, 1977). Entropies of fusion of organic compounds can thus be used as a model for entropy changes in protein folding. Nicholls et al. (1991) and Searle and Williams (1992) found $T\Delta S_{conf}$ to be -0.4 to -0.8 kcal·mol^{-1} per rotor at 300 K by considering fusion entropies of series of *n*-alkanes, alkyl carboxylic acids, and 2-methyl ketones, consistent with other results. Sternberg and Chickos (1994) estimated side-chain entropy by extending an empirical approach developed to model fusion entropy for small organic molecules. They calculated $T\Delta S_{conf}$ for 17 residue side chains (Table 1). The fusion entropy scale correlates well with each of the other scales listed in Table 1 and, importantly, gives results of the same magnitude. This confirms that taking the total ΔS_{config} as purely ΔS_{conf} is a good assumption.

The remainder approach

A final approach to ΔS_{conf} is to find each of the other terms that contribute to protein stability and attribute the difference from the experimental entropy change on folding to ΔS_{conf}. Privalov and Makhatadze (1993) estimated $T\Delta S_{conf}$ to be -3.0 kcal·mol^{-1} per residue at 300 K and Freire et al. (1993) found $T\Delta S_{conf}$ to be -0.9 kcal·mol^{-1} per residue in this way. The errors in this method are perhaps larger because they require the accurate estimation of all other terms.

Applications

Consideration of side-chain conformational entropies can help rationalize some aspects of protein structure. There is a tendency for residues with larger values of ΔS_{conf} to remain on the protein surface, where they will have higher entropy than if they were buried (Doig et al., 1993; Pickett & Sternberg, 1993). Shakhnovich and Finkelstein (1989) argued that the rate-determining step in protein folding is finding the correct side-chain rotamers.

A residue on the surface of a folded protein can be restricted in side-chain motion, though generally to a lesser extent than when it is buried. Creamer and Rose (1992, 1994) calculated side-chain entropies on the surface of α-helices, using Equation 2, where p_i is found by Monte Carlo simulation, and showed that these correlate well with experimentally measured helix preferences for eight nonpolar residues. Blaber et al. (1994) repeated this work for $\chi 1$ and $\chi 2$ for all 20 amino acids, finding p_i by surveying helices in crystal structures, and found a somewhat weaker correlation between ΔS_{conf} and helix preference. They argued that although side-chain conformational entropy goes some way to rationalize helix propensities, other factors also play a role (principally the hydrophobic effect).

Totrov and Abagyan (1994) incorporated side-chain entropy effects into an algorithm used to predict the structure of a protein/antibody complex. Jackson and Sternberg (1995) explored the inclusion of them to distinguish correctly from incorrectly docked protein/inhibitor complexes. Future applications of this work may include helping to understand the effects of point mutations on protein stability and in assessing correctly from incorrectly folded protein structures.

Conclusion

Comparison of each of these methods allows one to draw some general conclusions on the magnitude and importance of changes in side-chain conformational entropy. If the anomalous results of Privalov and Makhatadze (1993) are discounted, a consensus is reached that the cost of restricting side-chain motion ($T\Delta S_{conf}$) is ≈ -1 kcal·mol^{-1} per residue in protein folding or ≈ -0.5 kcal·mol^{-1} per rotamer. It is thus of considerable importance in protein stability.

The excellent agreement for ΔS_{conf} per side chain measured by several methods, using both empirical and theoretical approaches, suggests that the shape of each conformational well is indeed similar in the folded and unfolded states. In the folded state, a rotatable χ angle is merely restricted to fewer (often one) states. Changes in vibrational entropy can thus be safely ignored (i.e., $\Delta S_{vib} \approx 0$; $\Delta S_{config} \approx \Delta S_{conf}$) and only $\approx$25% of the absolute side-chain entropy is lost on burial in a folded protein.

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