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Entropy in Biological Binding Processes: Estimation of Translational Entropy Loss

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ABSTRACT **The loss of translational degrees of freedom makes an important, unfavorable contribution to the free energy of binding. Examination of experimental values suggest that calculation of this entropy using the Sackur–Tetrode equation produces largely overestimated values. Better agreement is obtained using the cratic entropy. Theoretical considerations suggest that the volumes available for the movement of a ligand in solution and in a complex are rather similar, suggesting also that the cratic entropy provides the best estimate of the loss of translational entropy.**

Key words: entropy, thermodynamics, binding energetics, translational entropy, macromolecular interactions

INTRODUCTION

The ability to predict binding affinities based on knowledge of the structure of a complex (e.g., protein–drug, protein–protein, protein–DNA) is an important goal in structural energetics. Such ability would be of great benefit in rational drug design as well as in redesigning protein structures. An important entropic contribution to the binding affinity comes from changes in translational degrees of freedom. It has been common to calculate this contribution based on the Sackur–Tetrode theory. In this paper we examine experimentally determined entropy changes and show that, after making reasonable estimates of other contributions to the entropy change, the remaining entropy change, attributable to changes in translational degrees of freedom, is significantly less than that calculated from the Sackur–Tetrode equation.

The binding affinity, K°, is directly related to the free energy of binding, ΔG°, through the relationship

$$\Delta G^\circ = -RT \ln K^\circ \qquad (1)$$

where R is the gas constant, T is the absolute temperature, and the superscript, °, indicates a *standard state free energy difference*. The standard state is defined by the pressure and the concentration units associated with K. In biological systems, K° is typically defined in molar concentration units.

The standard free energy of binding at any temperature T results from two contributions, the enthalpy change, ΔH°, and the entropy change, ΔS°:

$$\Delta G = \Delta H^\circ - T\Delta S^\circ. \qquad (2)$$

Note that ΔH° of an interaction is independent of the concentration units of the standard state. The entropy change associated with a binding reaction involving biological molecules can be divided into several terms. First is the solvent contribution arising from the restructuring of water, primarily around hydrophobic groups. Second is the configurational entropy change which reflects the restriction of rotational degrees of freedom about torsional angles of both the macromolecule and the ligand. Third is the entropy change arising from reduction in overall rotational degrees of freedom. Fourth, there is a term that reflects the decrease in translational degrees of freedom due to the reduction in the number of particles in solution upon binding. Only the last term is dependent on the choice of standard state. It is precisely the treatment of this term that has been the source of significant confusion.[1–5]

This paper discusses the evaluation of the translational entropy for processes occurring in aqueous solutions. The discussion is presented in three parts: first, methods to estimate changes in binding entropy are discussed briefly; second, three experimental examples are analyzed to assess which of the ways of estimating the translational contribution provides a better estimate; third, an analysis of the correct estimate of this entropic contribution from first principles is discussed.

The recent availability of accurate thermodynamic data for several processes permits an empirical evaluation of the various treatments existing in the literature. Many biological processes have an

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associated heat capacity change, ΔC_p, so that $\Delta H°$ and $\Delta S°$ are dependent on temperature:

$$\Delta H° = \Delta H°_R + \Delta C_p (T - T_R) \quad (3)$$

and

$$\Delta S° = \Delta S°_R + \Delta C_p \ln(T/T_R) \quad (4)$$

where T_R is any convenient reference temperature, typically 298 K. As with $\Delta H°$, ΔC_p is not dependent on the choice of concentration units of the standard state.

Recently, it has been demonstrated that both $\Delta H°$ and ΔC_p can be parameterized in terms of structural features, and that they can be predicted from crystallographic structures with reasonable reliability for both folding/unfolding transitions in globular proteins[6,7] and for the binding of a peptide hormone to an antibody.[1]

The solvent contribution to $\Delta S°$ can be calculated as[8,9]

$$\Delta S_{solv} = \Delta S^*_{solv} + \Delta C_p \ln(T/T_S^*) \quad (5)$$

where T_S^* is the temperature at which there is no solvent contribution to the hydrophobic entropy change and is equal to 385.15 K (112°C) and ΔS^*_{solv} includes protonation and electrostatic contributions. The total entropy value at 112°C is usually denoted by ΔS^* and is equal to the sum of the configurational, translational, and rotational entropy changes plus the polar contributions included in ΔS^*_{solv}. Since ΔC_p can be measured directly or reliably estimated if a high resolution structure is known, ΔS_{solv} can be well approximated for experimental conditions in which protonation or other electrostatic effects are absent or taken into account.

The configurational entropy change is not easily measured, but can be estimated theoretically[10,11] or from experimental studies.[12] It has been proposed recently[13] that the configurational entropy for protein folding/unfolding and protein associations could be written in terms of a minimum of three different types of contributions: (1) $\Delta S_{bu\rightarrow ex}$, the entropy change associated with the transfer of a side chain that is buried in the interior of the protein to its surface; (2) $\Delta S_{ex\rightarrow u}$, the entropy change gained by a surface exposed side chain when the peptide backbone unfolds; and (3) ΔS_{bb}, the entropy change gained by the backbone itself upon unfolding. The total configurational entropy change can be written as

$$\Delta S_{conf} = \sum_i \Delta S_{ex\rightarrow u,i} + \sum_j \Delta S_{bu\rightarrow ex,j} + \Delta S_{bb} \quad (6)$$

where the summation i runs over all amino acid side chains and the summation j runs only over those amino acids that are buried.

As a first approximation, the value of $\Delta S_{ex\rightarrow u,i}$ can be equated to the side chain entropy change associated with a helix-to-coil transition. Recently, Creamer and Rose[11] estimated side chain contributions to the configurational entropy change of helix-to-coil transitions in isolated peptides using Monte Carlo simulations. According to their study, the average side chain contribution for the amino acids studied (Ile, Leu, Met, Phe, Trp, Tyr, Val) is on the order of 0.74 cal K^{-1} mol^{-1}. This value is close to the one derived previously by Némethy et al.[10] (0.5 cal K^{-1} mol^{-1}). Creamer and Rose calculated also the absolute configurational entropy of side chains in the α-helix, which ranges between 0.2 and 4.7 cal K^{-1} mol^{-1}, the average being 2.8 ± 1.7 cal K^{-1} mol^{-1} for the amino acids studied. If it is assumed that the side chains buried in the interior of the protein have zero configurational entropy, then those entropy values can be considered to approximate the configurational entropy change of transferring a side chain from the interior of the protein to its surface, $\Delta S_{bu\rightarrow ex}$.

The backbone entropy is a function of the peptide chain length and composition, as well as the presence of disulfide bridges or other covalent bonds in the backbone. In the absence of covalent links, the backbone entropy is primarily a function of the steric hindrances imposed by the side chains on the rotational degrees of freedom of the peptide chain. This entropy term is maximal for glycine, it decreases rather dramatically for alanine and continues to decrease for larger side chains. At the present time no accurate estimates for these contributions are available. It has been roughly estimated that glycine should contribute on the order of 5 cal K^{-1} mol^{-1} and that this value should drop to about 3 cal K^{-1} mol^{-1} for alanine.[1,10]

The above estimates for the configurational entropy contributions are consistent with the weighted average overall value of 4.3 cal K^{-1} $(mol\text{-}res)^{-1}$ obtained from the thermodynamic database for the folding/unfolding of monomeric proteins.[7-9] For monomolecular folding/unfolding reactions, this average value per residue includes contributions both from the peptide backbone and from amino acid side chains weighted according to the amino acid composition and is experimentally given by ΔS^*, the entropy change evaluated at T_S^* after correction for protonation and electrostatic contributions.

The sum of the configurational and solvent related entropy terms can be considered to represent the *unitary entropy* change.[14] (Unitary because it depends only on the state of a single molecule.) The change in translational degrees of freedom due to the reduction in the number of particles in solution upon binding is reflected in the mixing entropy. The change in mixing entropy for the formation of 1 mol of complex in a binding reaction with the following stoichiometries:

$$iA + jB + \cdots + kC \leftrightarrow P \quad (7)$$

is given by

$$\Delta S_{mix} = (i + j + \cdots + k - 1)R \ln X + R(i \ln i + j \ln j + \cdots + k \ln k) \quad (8)$$

if the solution is sufficiently dilute to be considered ideal. In Eq. (8), X is the mole fraction of complex. Equation (8) is a generalized expression for the so-called *cratic entropy* as discussed by Kauzmann.[14] If the concentrations that define the equilibrium constant are expressed in molar units then the entropy change corresponds to the formation of 1 mol of product in 1 liter. In this case $X = 1/55.5$. Accordingly, for a simple binding reaction of the form $A + B \leftrightarrow P$, $\Delta S_{mix} = -8$ cal K^{-1} mol^{-1}. On the other hand, for a reaction of the form $A + 2B \leftrightarrow P$, $\Delta S_{mix} = -13.2$ cal K^{-1} mol^{-1} of complex formed. It must be noted also that the binding equilibrium constant mentioned above is the macroscopic equilibrium constant for the overall binding process. For those cases in which the intrinsic or microscopic binding constant per site is used, Eq. (8) is still valid except that the reaction is defined as corresponding to the formation of 1 mol of bound sites from 1 mol of ligand and 1 mol of binding sites.

Some authors have treated the change in translational degrees of freedom associated with binding in terms of the Sackur–Tetrode equation (see for example [2,15]). This equation is evaluated from the quantum states of a particle in a box and assumes that the particle has zero volume and no intermolecular interactions. For molecules with molecular weights similar to those of proteins, the decrease in translational entropy is predicted to be near -25 cal K^{-1} mol^{-1} for the formation of a bimolecular complex, a value three times larger than the cratic entropy term. The validity of these approaches can be tested by comparing experimentally determined quantities, once estimates of the remaining entropic contributions have been made. We will examine three examples: the aqueous dissolution of cyclo-(glycylglycine) [c(GG)], the binding of angiotensin II (A2) to a monoclonal antibody, and the effect of dimer formation on the stability of the coiled-coil protein, GCN4.

EXAMPLE 1: AQUEOUS DISSOLUTION OF A CYCLIC DIPEPTIDE

The aqueous dissolution of cyclic dipeptides has been studied by direct calorimetric methods.[16] The $\Delta S°$ for c(GG) dissolution at 298 K is 17 cal K^{-1} mol^{-1} using a 1 M standard state.[17] Because the ΔC_p of dissolution is nearly zero for this compound, no solvent contributions are considered. Additionally, the lack of side chains and the closed ring result in minimal rotamer contributions to $\Delta S°$. Thus the 17 cal K^{-1} mol^{-1} can be considered as the sum of the dilution and the mixing (cratic) entropy change, both of which define the change in translational entropy, ΔS_{trans}. The cratic entropy is 8 cal K^{-1} mol^{-1} for a unit molar reference state. The difference of 9 cal K^{-1} mol^{-1} can be attributed to the gain in translational degrees of freedom upon dilution and the gain in rotational degrees of freedom.

Application of the Sackur–Tetrode equation (MW = 114 Da) yields $S_{trans} = 34$ cal K^{-1} mol^{-1} for c(GG) in the saturated solution. This value can be taken as ΔS_{trans} since the translational entropy in the crystal is very small. It would seem that the Sackur–Tetrode equation overestimates the translational entropy of a cyclic dipeptide in solution. Searle et al.[15] arrived at the same conclusion; however they did not correct for the standard state of the c(GG) dissolution data and they assumed a sizable hydrophobic contribution to $\Delta S°$, inconsistent with the small value of ΔC_p.

EXAMPLE 2: BINDING OF A HORMONE TO AN ANTIBODY

The binding of the flexible hormone angiotensin II to a monoclonal antibody has recently been studied calorimetrically.[1] The experimentally determined $\Delta S°$ is 7 cal K^{-1} mol^{-1} at 303 K, using a 1 M standard state and the microscopic binding constant. The ΔC_p for the reaction is -250 cal K^{-1} mol^{-1} yielding an estimate of the solvent contribution to $\Delta S°$ [by Eq. (5)] of 60 cal K^{-1} mol^{-1}. Angiotensin II has eight residues, giving a configurational entropy change upon binding of approximately -34 cal K^{-1} mol^{-1}. The configurational entropy change due to the freezing of the antibody side chains in the binding pocket has been roughly estimated[1] as -17 cal K^{-1} mol^{-1}. The sum of the solvent and the configurational contributions to $\Delta S°$ is 9 cal K^{-1} mol^{-1}. The cratic term is -8 cal K^{-1} mol^{-1}, giving a sum total of 1 cal K^{-1} mol^{-1}. This value is reasonably close to the experimental value of 7 cal K^{-1} mol^{-1} (in our original paper[1] the cratic entropy was incorrectly treated). In contrast, the translational contribution calculated using the Sackur–Tetrode equation is on the order of -25 cal K^{-1} mol^{-1} for A2 (MW = 1000 kDa)[15] which would lead to an overall $\Delta S°$ of -16 cal K^{-1} mol^{-1}.

EXAMPLE 3: EFFECT OF DIMER FORMATION ON GCN4 STABILITY

Recently, the stability of GCN4, a two-stranded α-helical coiled-coil composed of two identical 35-residue peptide chains, has been measured as a function of concentration by both differential scanning calorimetry and CD spectropolarimetry.[18] As expected, the stability of the coiled-coil is concentration dependent since the unfolding process is coupled to the dissociation of the two strands. The analysis of the data was performed as described[18] using the equations for a monomer–dimer equilibrium. The experimentally determined $\Delta S°$ for the formation of the coiled-coil at 70°C was found to be

-84.7 cal K^{-1} mol^{-1} normalized to the monomer concentration.

From the analysis of the structure, the solvent contribution to $\Delta S°$ at 70°C is estimated to be 46 cal K^{-1} mol^{-1}. This positive entropy arises primarily from the burial of the largely hydrophobic interface of the coiled-coil (leucine zipper). The configurational $\Delta S°$, arising from the restriction in backbone and side chain degrees of freedom, is approximated as -130 cal K^{-1} mol^{-1}. There is an additional electrostatic contribution to $\Delta S°$ due to the formation of salt bridges across the dimer interface estimated as 7 cal K^{-1} mol^{-1} using the Tanford–Kirkwood equation.[19] The cratic term, normalized to the monomer is -4 cal K^{-1} mol^{-1}, yielding a sum total of -81 cal K^{-1} mol^{-1}, again very close to the experimental value of -84.7 cal K^{-1} mol^{-1}. In contrast, the translational contribution calculated using the Sackur–Tetrode equation is on the order of -25 cal K^{-1} mol^{-1} which again leads to much poorer agreement with the experimental values.

DISCUSSION

The three examples above illustrate that the translational contributions to $\Delta S°$ for biological binding reactions in aqueous solutions appear to be better accounted for by the cratic entropy term as discussed by Kauzmann than the Sackur–Tetrode equation derived for ideal gases. This is, perhaps, not too surprising if the assumptions involved in the derivation of the Sackur–Tetrode equation are taken into consideration. First is the assumption that a molecule in solution can be approximated by a flat potential within the confines of the container, i.e, the box. While such an assumption is valid for an ideal gas where no interactions occur between particles, it does not hold for solutions where interactions between solute and solvent are dominant. Second, the derivation also assumes that the volume which defines the standard state (1000 ml for a 1 M solution) is freely accessible to the solute in the same way that 22,400 ml are accessible to a mole of gas at 1 atm and 273 K. This assumes that the particles are mathematical points occupying no volume. Just as the ideal gas law cannot be applied to solutions where much of the volume is excluded, the gas entropy equations become invalid where real volumes must be considered.

In principle, it is possible to estimate the actual volume within which the individual molecules are free to move (i.e., the volume in which the Sackur–Tetrode equation applies) in the bound complex and when they are free in solution. The movement of the bound molecule can be estimated from the values of the temperature factors (B) in X-ray determined structures of complexes. Typical B values are 15–30 $Å^2$ corresponding to rms displacements of 0.4–0.6 Å* or rms volumes of 0.03–0.22 $Å^3$. For molecules free in solution, the average linear distance $<l>$ that the center of a molecule can freely traverse at any time can be estimated as suggested by Rice[20] for the molecules in a liquid. Rice suggested that the distance available for the movement of a molecule is the linear distance between molecules minus the diameter of the molecule, that is $(V/N)^{1/3} - d$. In this expression, V is the volume occupied by N molecules and d is the diameter of a molecule. For molecules in aqueous solution, $<l>$ for the reacting molecules should be similar to that of the water molecules. For water $<l> = 3.1 - 2.8 = 0.3$ Å, a figure not larger than that for the molecules in the complex, assuming their mobility is similar to that in the crystal structure. Thus, transfer of the ligand from the solvent to the complex does not change appreciably the volume in which the ligand is free to move under a constant potential and, as such, this effect should not contribute significantly to the entropy change. The major entropic difference between the two states is then the cratic entropy, that is, the entropy contribution arising from the mixing of solute and solvent molecules. This term is the equivalent for solutions of the "communal" entropy ($\Delta S_{communal} = R$) used in describing the transition from solid to liquid. The value of the communal entropy is in excellent agreement with the entropies of melting of plastic crystals (crystals with a purely translational melting transition) that are in the range of 1–3 cal K^{-1} mol^{-1}.[21]

Williams and co-workers have also noted that the translational contributions to the entropy are overestimated using the classical approach, and have suggested that these terms are better estimated from entropies of vaporization.[5] The analysis presented here suggests that the cratic entropy provides a better quantitative account of the experimental results.

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*Correction for overall vibrations and translations of the whole molecule yields a value of approximately 0.2–0.3 Å.

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