The Concentration Dependence of the ΔS Term in the Gibbs Free Energy Function: Application to Reversible Reactions in Biochemistry

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As cellular conditions change, many enzyme-catalyzed reactions may proceed in either the forward or reverse direction. In introductory biochemistry, the most important application of thermodynamics is the consideration of ΔG to determine the directionality of a reaction. This idea is central to understanding coupled reactions and the role of ATP as a form of free energy currency in biological systems. A standard biochemistry curriculum includes two expressions for the change in free energy of a reaction,

$$\Delta G = \Delta H - T\Delta S \tag{1}$$

$$\Delta G = \Delta G^{\circ\prime} + RT \ln\left(\frac{[\mathrm{C}]^c[\mathrm{D}]^d}{[\mathrm{A}]^a[\mathrm{B}]^b}\right) \tag{2}$$

in which G, H, and S represent the thermodynamic functions for free energy, enthalpy, and entropy of the system, respectively, $\Delta G^{\circ\prime}$ represents the standard free energy change at pH 7.0 (the biological standard state convention indicated by the prime), T is the absolute temperature, R is the gas constant, and the concentration terms refer to the generic reaction $a\mathrm{A} + b\mathrm{B} \rightleftharpoons c\mathrm{C} + d\mathrm{D}$ in which the lower case letters are the stoichiometric coefficients. Although the "super bar" notation is not usually used in biochemistry texts, ΔG, ΔH, and ΔS are intensive functions given on a per mole basis, and they use the chemists' delta *(1)* for rate of infinitesimal change, sometimes written as Δ_r with the subscript indicating reaction *(2)*.

Equations 1 and 2 allow one to determine whether a reaction proceeds in the forward direction (if ΔG is negative), the reverse direction (if ΔG is positive), or is at equilibrium (if $\Delta G = 0$) for a given set of reactant and product concentrations. Biochemistry textbooks ordinarily present these two equations in the same chapter, but rarely comment on their relationship to one another. Both equations characterize reversible reactions that tend toward equilibrium, but eq 1, the Gibbs free energy equation, is the more difficult to grasp conceptually. Homework problems involving the calculation of ΔG typically make use of eq 2 exclusively, which can make the Gibbs equation seem virtually irrelevant. Experience with eq 2 enables students to understand that ΔG varies with reaction progress as reactant and product concentrations change. Equations 1 and 2 are both correct and each describes the same ΔG, so they must be consistent with one another. On this basis, students can "know" that ΔG in eq 1 must also be concentration dependent, but to *understand* why this is so, they must recognize that at least one of the terms that defines ΔG in eq 1 is concentration dependent.

Concentration Dependence in Equation 1

For biochemical processes that occur at constant temperature and pressure, the directionality of a reaction is determined solely by the concentrations of the reactants and products that are present. Equation 2 contains explicit concentration terms and is therefore obviously concentration-dependent, whereas eq 1 lacks such terms and could appear, superficially, to be concentration independent. ΔG in eq 1 is defined in terms of ΔH, which is essentially independent of concentration, and ΔS, whose concentration dependence might not be recognized by students who have not studied physical chemistry.

ΔS incorporates both concentration-dependent and concentration-independent entropy terms. Often, the concentration-independent determinants of ΔS are more familiar to biochemistry students. For example, most students know that the local (system) entropy decreases when differences in translational or rotational freedom cause products to be more constrained than the reactants from which they were created. One easy way to recognize this type of entropic effect is by considering reaction stoichiometry. In fact, many biochemistry textbooks explain ΔS in this way. One of the most widely used texts employs the gas-phase reaction $2\mathrm{H}_2 + \mathrm{O}_2 \rightarrow 2\mathrm{H}_2\mathrm{O}$ to illustrate the thermodynamic concepts that are expressed in the Gibbs free energy function. The entropic effect of the reaction is explained: "If the temperature of the system is held constant, the entropy of the system decreases because three moles of two differing reactants have been combined to form two moles of a single product" *(3)*. Another popular text that uses the same example comments that "two water molecules, each of whose three atoms are constrained to stay together, are more ordered than the three diatomic molecules from which they formed" *(4)*. Generalized statements equating stoichiometry with the sign of ΔS are encountered in other leading texts as well, as in "Whenever a chemical reaction results in an increase in the number of molecules …molecular disorder, and thus entropy, increases" *(5)*.

These stoichiometric explanations for the sign of ΔS are useful, but they do nothing to convey the concentration dependency of ΔS, because stoichiometry remains invariant even as reactants are consumed and products accumulate. If the partial molar quantities ΔS and ΔH were each thought to be constant during a reaction, it would be difficult to see how eq 1 could be used to calculate values of ΔG that vary as the reaction progresses. Therefore, it is appropriate to mention entropy of mixing, the concentration-dependent component of ΔS, when teaching the Gibbs free energy function.

Entropy of Mixing

Entropy is a measure of the number of microstates of a system that are equivalent in energy and macroscopically indistinguishable *(6, 7)*. The entropy is proportional to the logarithm of the number of indistinguishable microstates. The entropy of mixing (ΔS_{mixing}) reflects the change in the number of equivalent microstates that become available upon mixing of two or more chemical species. The traditional illustration of ΔS_{mixing} involves two ideal gases at the same pressure and temperature kept separated by a partition. If the partition is removed, spontaneous mixing occurs, and the entropy of the system increases by the quantity ΔS_{mixing}. The volume accessible to each gas increases when the partition is removed, so the partial pressure of each gas becomes lower after mixing while the total pressure remains constant. It is the dispersal of each species within a larger volume (i.e., their dilution), rather than the mixing per se, that fundamentally affects entropy *(8, 9)*. But entropy of mixing is established terminology, perhaps because mixing conjures up a more vivid mental image than dilution. For biochemical processes that occur in aqueous solutions, the dilution of solutes that occurs as a result of mixing is conceptually analogous.

The entropy of mixing, ΔS_{mixing}, is designated using a delta symbol because it is the difference between the entropy of the mixed state and the entropy of the corresponding unmixed state (i.e., the change in entropy that occurs upon removing the partition, in the previous example). The value of ΔS_{mixing} is

$$\Delta S_{\text{mixing}} = -R\left(x_A \ln x_A + x_B \ln x_B + x_C \ln x_C + \ldots\right) \quad (3)$$

where x_A, x_B, and x_C denote mole fractions of species A, B, and C, and ΔS_{mixing} is expressed per mole based on the total number of moles present in the mixture. The discussion of entropy of mixing has focused thus far on the mixing of distinct, preexisting species that are chemically inert, but mixing and unmixing effectively occur during the course of a chemical reaction as a consequence of chemical transformation. A system initially containing only reactants becomes more mixed as product species form, and the entropy of mixing increases accordingly. If the reaction goes nearly to completion, unmixing occurs as the system moves toward a final state consisting predominantly of product. At any point during the course of the reaction, the instantaneous rate of change in ΔS_{mixing} ($\Delta\Delta S_{mixing}$)[1] describes the effect of further reaction progress on the value of ΔS_{mixing}. Using concentration terms instead of mole fractions, the expression for $\Delta\Delta S_{mixing}$ obtained from the derivative of eq 3 is:[2]

$$\Delta\Delta S_{\text{mixing}} = -R\ln\left(\frac{[\text{C}]^c[\text{D}]^d}{[\text{A}]^a[\text{B}]^b}\right) \quad (4)$$

ΔS_{mixing} is always equal to or greater than 0; it is zero for a completely unmixed state, and varies with reaction progress, reaching its maximum value when reactant and product species become maximally mixed. $\Delta\Delta S_{mixing}$, corresponding to the slope of the plot of ΔS_{mixing} versus extent of reaction, is positive during mixing, negative during unmixing, and zero at the point during a reaction at which ΔS_{mixing} is maximal.

Equivalence of Equations 1 and 2

ΔS is given by the sum of the change in nonmixing entropy (the standard reaction entropy) and the change in entropy of mixing (expressed in eq 4):

$$\Delta S = \Delta S^\circ + \left\{-R\ln\left(\frac{[\text{C}]^c[\text{D}]^d}{[\text{A}]^a[\text{B}]^b}\right)\right\} \quad (5)$$

$$\Delta S = \begin{matrix}\text{change in}\\ \text{nonmixing}\\ \text{entropy}\end{matrix} + \begin{matrix}\text{change in}\\ \text{entropy of}\\ \text{mixing}\end{matrix}$$

This relationship can be used to show that eq 2, the "workhorse" equation in general biochemistry, is essentially a simple algebraic rearrangement of eq 1, the Gibbs free energy equation. Substitution of eq 5 into eq 1 gives

$$\Delta G = \Delta H - T\left\{\Delta S^\circ - R\ln\left(\frac{[\text{C}]^c[\text{D}]^d}{[\text{A}]^a[\text{B}]^b}\right)\right\} \quad (6)$$

$$\Delta G = \left(\Delta H - T\Delta S^\circ\right) + RT\ln\left(\frac{[\text{C}]^c[\text{D}]^d}{[\text{A}]^a[\text{B}]^b}\right) \quad (7)$$

For an ideal solution, $\Delta H = \Delta H^\circ$, so the first term can be replaced according to the formula $\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ$ to give,

$$\Delta G = \Delta G^\circ + RT\ln\left(\frac{[\text{C}]^c[\text{D}]^d}{[\text{A}]^a[\text{B}]^b}\right) \quad (8)$$

which is the same as eq 2 if pH = 7 so that $\Delta G^\circ = \Delta G^{\circ\prime}$.

Equation 5 shows explicitly that entropy change involves both concentration-dependent and concentration-independent contributions. Entropic effects due to mixing, given by the second term in eq 5, are concentration dependent. In the initial phase of a reaction, when [reactants] >> [products], the term $-R\ln([\text{C}]^c[\text{D}]^d/[\text{A}]^a[\text{B}]^b)$ is positive and contributes toward increasing ΔS, which indicates that concentration-dependent entropic effects favor the forward reaction during this early phase (note that the overall ΔS could still be positive or negative). The logarithmic term becomes zero at the point at which the mixing of reactant and product species is maximal, which occurs when [reactants] = [products] for a simple unimolecular conversion, and corresponds to the point at which $[\text{C}]^c[\text{D}]^d/[\text{A}]^a[\text{B}]^b = 1$ in the general case. Continuation of forward reaction progress beyond this point causes the $-R\ln([\text{C}]^c[\text{D}]^d/[\text{A}]^a[\text{B}]^b)$ term to become negative, which indicates that the number of mixing-related microstates available is decreasing. Although concentration-dependent entropic effects tend to drive a reaction toward its position of maximum mixing, this point does not correspond to equilibrium. Equilibrium is determined by the interplay of enthalpy, temperature, and the complete ensemble of entropic effects, as expressed in eq 6.

Entropy Changes during Glucose Conversion

The concentration dependence of the ΔS term in the Gibbs free energy function can be explained in the classroom by examining a simple, idealized reaction that is familiar to biochemistry students. Consider the anomeric conversion of glucose in solution: α-D-glucose(aq) ⇌ β-D-glucose(aq). Starting with freshly dissolved α-D-glucose as the reactant, the reaction proceeds spontaneously to produce β-D-glucose, via the open-chain aldehyde intermediate. Net production of β-D-glucose continues until equilibrium is reached. The uncatalyzed reaction is fairly slow, requiring over an hour to reach equilibrium at neutral pH. At 25 °C, the equilibrium solution contains 36% α-D-glucose and 64% β-D-glucose *(10).* The quantity of the open-chain intermediate, < 0.003% *(11),* can be considered to be negligible for purposes of this discussion. The reaction is slightly exothermic, with standard enthalpy $\Delta H^\circ_{\alpha\rightarrow\beta} = -1.15$ kJ mol^{-1} *(12).* This value includes solute-solvent interactions, such as H-bonds between glucose hydroxyl groups and water molecules, and shows that enthalpy favors the formation of the β-anomer of glucose, which has its C-1 and C-2 hydroxyl groups located on opposite sides of the ring. The enthalpy relationship between reactant and product is shown in Figure 1.

The reaction is reversible, and if the concentration of β-D-glucose were to exceed its equilibrium value of 64%, the reaction would proceed in the reverse direction. The reverse reaction could be promoted by adding β-anomer to the system or by depleting the α-anomer. The forward reaction (from α-D-glucose to the equilibrium state) and the reverse reaction (from β-D-glucose to the equilibrium state) are depicted schematically in Figure 2, which emphasizes that the direction of the reaction A ⇌ B is determined by the relative concentrations of A (α-D-glucose) and B (β-D-glucose).

Figure 2 depicts seven "snapshots" of the reaction, captured at various reactant:product concentration ratios. The snapshots were chosen to give a symmetrical picture of the reaction at roughly similar intervals and to include four landmark positions of special interest (pure reactant, pure product, equimolar reactant and product, and the equilibrium point). The concentrations of reactants and products at each stage of the reaction determine the free energy of the system at that point. The system has the lowest free energy at equilibrium, when the mole fractions are 0.36 for A and 0.64 for B. In Figure 2, the arrowheads in the row labeled "Rxn direction (ΔG)" are oriented to indicate reaction steps that decrease free energy.

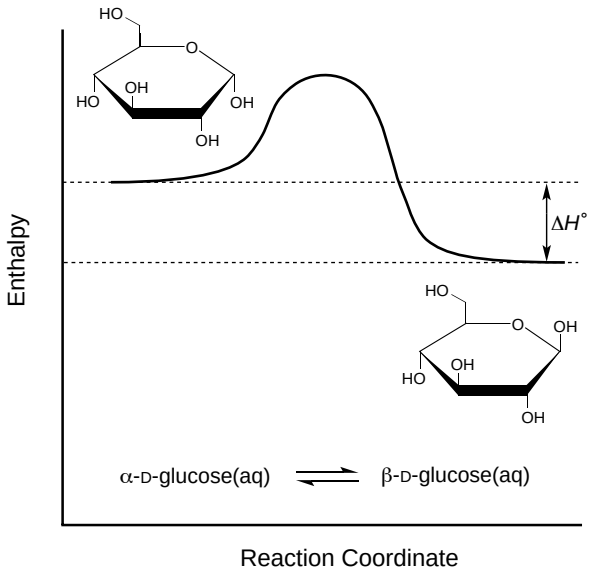

Figure 1. The formation of the β-anomer of glucose is exothermic. Solvated α-D-glucose (left) has greater enthalpy than β-D-glucose (right). Hydrogen atoms are not shown (except hydroxyl), and the local minimum for the open-chain intermediate has been omitted for clarity.

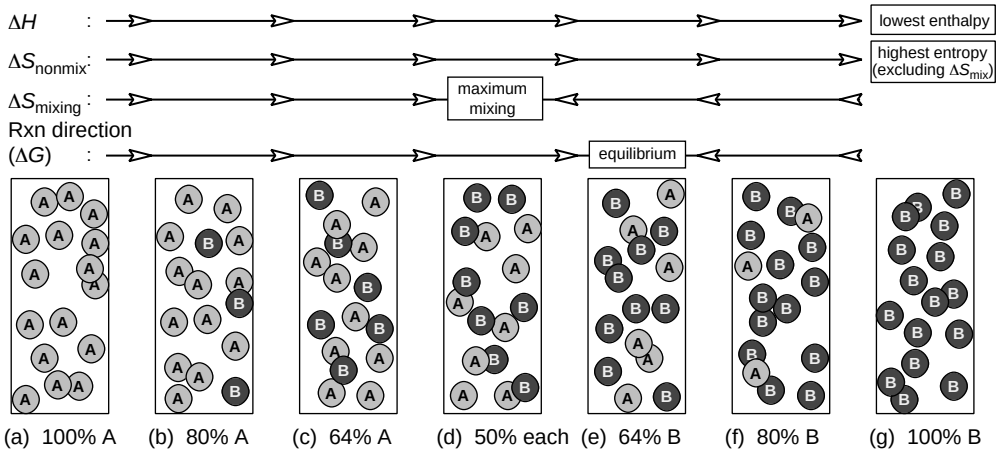

Figure 2. Concentration determines reaction directionality. The reaction α-D-glucose(aq) ⇌ β-D-glucose(aq) moves in the forward direction (panels a, b, c, and d) or reverse direction (f and g) toward equilibrium (e). α-D-glucose and β-D-glucose molecules are labeled A and B, respectively. Arrowheads indicate the actual reaction direction (row ΔG), and the direction of the contribution to ΔG provided by the constituent thermodynamic functions (rows ΔH, $\Delta S_{\text{nonmixing}}$, and ΔS_{mixing}).

The reaction α-D-glucose(aq) ⇌ β-D-glucose(aq) is exothermic, so enthalpy favors the forward reaction regardless of reactant and product concentrations. For dilute solutions, ΔH_{mixing} is negligible and $\Delta H = \Delta H°$. Therefore, at any point during the reaction, enthalpy provides a contribution of −1.15 kJ mol^{-1} to ΔG, which encourages the forward reaction. This tendency due to enthalpy is indicated by right-pointing arrowheads at every position in the row labeled "ΔH" in Figure 2. Likewise, nonmixing entropy provides an invariant contribution that also favors the forward reaction at all concentrations,[3] as shown by the right-pointing arrowheads in the "$\Delta S_{nonmixing}$" row in Figure 2. The standard reaction entropy $\Delta S°_{\alpha\rightarrow\beta}$ = +0.94 J K^{-1} mol^{-1} (see endnote 4), so the magnitude of the driving force (expressed as free energy change) represented by these arrowheads is (−298 K)(+0.94 J K^{-1} mol^{-1}) = −280 J mol^{-1} or −0.28 kJ mol^{-1}. The positive value for $\Delta S°_{\alpha\rightarrow\beta}$ indicates that there is a small increase in the nonmixing entropy of the system (solute plus solvent) as the β-anomer is formed. Unlike ΔH and $\Delta S_{nonmixing}$, ΔS_{mixing} varies with concentration. When [α-D-glucose] = [β-D-glucose], the entropy of mixing is maximal and the free energy of mixing is at its minimum (panel d in Figure 2). Arrowheads for ΔS_{mixing} in Figure 2 are oriented to indicate reaction steps that increase the entropy as a result of mixing.

The reaction α-D-glucose(aq) ⇌ β-D-glucose(aq) proceeds until the system reaches its minimum value of Gibbs free energy at the equilibrium point (panel e in Figure 2). One way to recognize the free energy minimum is to compare the free energy of the system at the start of the reaction (when no product has yet been formed) to its free energy at later stages. Reaction progress is described by ξ, the extent of reaction, which varies from 0 to 1. In the present example, ξ is the same as the mole fraction of the product, β-D-glucose. The change in the free energy that results from reaction progress from the initial state (ξ = 0) to any intermediate state (ξ = i) is $G_i - G_0$, and the minimum value of $G_i - G_0$ occurs at equilibrium. This difference, $G_i - G_0$, represents the change in G over a finite interval, and it is distinct from ΔG, which refers to the derivative of G with respect to ξ *(1, 13)*. $G_i - G_0$ is given by the equation,

$$G_i - G_0 = \xi\Delta G° + \left(-T\Delta S_{mixing}\right) \qquad (9)$$

in which the first term indicates the nonmixing contribution due to $\Delta H - T\Delta S_{nonmixing}$ and the second term accounts for the effect of mixing. Table 1 shows the net change in G as the reaction progresses to reach each of the seven reaction snapshot positions shown in Figure 2. These values were determined using eq 9 with $\Delta G°$ = −1.43 kJ mol^{-1}, T = 298 K, and ΔS_{mixing} calculated from the mole fractions according to eq 3. Table 1 shows that the equilibrium state (panel e), consisting of 64% β-D-glucose, is about 0.10 kJ mol^{-1} lower in energy than an equimolar mixture of α- and β-anomers.

It is more convenient to find the free energy minimum during a reaction by setting ΔG = 0 in eqs 1 or 2. This identifies the point at which the slope is zero in the plot of G versus ξ. Although eq 2 is most often used in this context, it is useful to note that eq 2 is little more than a rearrangement of eq 1, as shown by eqs 6–8. Equation 1 can be used to solve for the equilibrium ratio of reactant and product at 298 K, by setting ΔG = 0, expanding the expression for ΔS as shown in eq 5, and using $\Delta H = \Delta H°$ = −1.15 kJ mol^{-1} and $\Delta S°$ = +0.94 J K^{-1} mol^{-1}. After these substitutions, the equation simplifies to ln([β-D-glucose]/[α-D-glucose]) = 0.577, and taking the antilog of this gives [β-D-glucose]/[α-D-glucose] = 1.78, which is equivalent to saying that the equilibrium mole fractions are 0.64 and 0.36, respectively.

A Qualitative Approach: Pictorial Representation

A quantitative appreciation of the interaction of the ΔH, ΔS_{mixing}, and $\Delta S_{nonmixing}$ terms in determining free energy change is an important objective for physical chemistry students *(14)*. However, a course in physical chemistry is rarely a prerequisite for general biochemistry. It is beyond the scope of most introductory biochemistry courses to analyze entropy contributions in a quantitative manner, yet some feel for the concentration dependence of ΔS in eq 1 is necessary to avoid misconceptions about reaction reversibility. It is helpful to have an intuitive argument for the dependence of ΔS on concentration. A simple pictorial example is an efficient way to introduce the concept to an undergraduate biochemistry audience when time constraints preclude a more rigorous presentation of the topic. A three-step argument for the existence of a concentration-dependent (mixing) contribution to en-

Table 1. Change in Free Energy in the Glucose Conversion Reaction

Panel in Figure 2	Mole Fraction A	Mole Fraction B	$\xi\Delta G°$/ (kJ mol^{-1}) (nonmixing)	$-T\Delta S_{mix}$/ (kJ mol^{-1}) (mixing)	$G_i - G_0$/ (kJ mol^{-1}) (total)
a	1.00	0.00	0.00	0.00	0.00
b	0.80	0.20	-0.29	-1.24	-1.53
c	0.64	0.36	-0.51	-1.62	-2.13
d	0.50	0.50	-0.71	-1.72	-2.43
e	0.36	0.64	-0.91	-1.62	**-2.53**
f	0.20	0.80	-1.14	-1.24	-2.38
g	0.00	1.00	-1.43	0.00	-1.43

tropy that can help to explain why reactions go to equilibrium instead of going to completion is shown in Figure 3.

The effect of reaction progress on mixing is complicated by the concomitant phenomenon of chemical transformation. Textbooks often illustrate mixing using inert gases as a way to simplify the presentation, but this approach sidesteps the important point that mixing (or unmixing) can occur as a direct consequence of chemical change. Mixing that occurs as a result of chemical transformation is the basis for the concentration dependence of ΔS during a reaction. For teaching purposes, it is helpful to highlight the similarity between the simpler case, mixing of inert species, and the more complex case, mixing that occurs as the result of reaction. The upper panel of Figure 3 shows pure reactant and product species combining to form a mixture, which is an entropically favorable process that occurs spontaneously. The model is simplified at this point by delaying consideration of chemical reactivity, so that the concentrations of reactant, A, and product, B, are unchanged during the process. It is a small step to recognize that the reverse of this process (unmixing of A and B) is opposed by entropy, as illustrated in panel b of Figure 3. Finally, panel c shows that the chemical conversion of reactant into product can result in the creation of a state that is increasingly unmixed and therefore disfavored by entropy. The visual similarity between panels b and c allows this conclusion to be recognized more easily. As a reaction proceeds beyond its mixing midpoint, the entropy due to mixing decreases. The entropy costs become progressively higher as product accumulates and the system becomes increasingly unmixed. In the case of the reaction α-D-glucose(aq) ⇌ β-D-glucose(aq), forward reaction progress cannot occur beyond 64% B because of the uncompensated loss of entropy that would come from additional unmixing.

As emphasized in Figure 2, equilibrium represents the balance between the invariant value of $\Delta H - T\Delta S_{\text{nonmixing}}$, which favors the reaction in a single direction, and ΔS_{mixing}, which favors a reaction mixture containing substantial quantities of both reactants and products. The simple diagrams presented here are intended to provide biochemistry students with an intuitive and non-mathematical argument for the concentration dependence of the ΔS term in the Gibbs free energy equation. With a minimum investment of lecture time, the applicability of this equation to reversible reactions that approach equilibria can be made clearer. For those interested in incorporating these diagrams into their teaching, electronic versions of the figures are available in the Supplemental Material.[W]

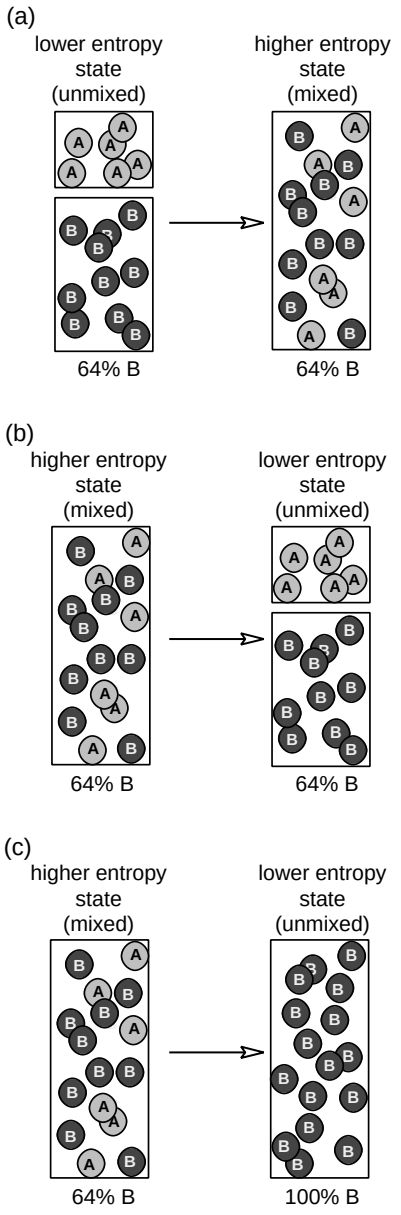

Figure 3. Reactant and product concentrations affect entropy. (a) A and B mix spontaneously, an entropically favorable process. (b) Entropy opposes unmixing, the reverse of the process shown in panel a. (c) If the reaction A ⇌ B were to proceed to completion, the mixed state (64% B at equilibrium) would be converted to an unmixed state (100% B at completion). Conceptually, this unfavorable process resembles that shown in panel b.

[W]Supplemental Material

Electronic versions of the figures are available in this issue of *JCE Online*.

Notes

1. By convention, the Δ symbol in ΔS_{mixing} indicates a difference, but the first Δ in $\Delta\Delta S_{\text{mixing}}$ is intended as a chemists' delta *(1)* indicating the derivative.

2. The simplest derivation of eq 4 from eq 3 is for the two-component mixture that results when aA + bB ⇌ cC + dD takes the form A ⇌ C. In this case, $x_C = \xi$ (the extent of reaction), and $x_A = 1 - \xi$. Substituting these expressions into eq 3 gives $\Delta S_{\text{mixing}} = -R[(1-\xi)\ln(1-\xi) + \xi\ln\xi]$. Taking the derivative gives $d\Delta S_{\text{mixing}}/d\xi = -R\{(-1)\ln(1-\xi) + (1-\xi)(-1)[1/(1-\xi)] + (1)\ln\xi + \xi(1/\xi)\} = -R[-\ln(1-\xi) - 1 + \ln\xi + 1] = -R[\ln\xi - \ln(1-\xi)] = -R\ln[\xi/(1-\xi)] = -R\ln(x_C/x_A) = -R\ln([C]/[A])$. Equation 4 applies to ideal solutions in which solute activities are given by molar concentrations.

3. It is a coincidence that, in this particular example, enthalpy and nonmixing entropy each favor the forward direction. However, even when these two factors act in opposition, the sum (ΔH –

$T\Delta S_{nonmixing}$) will be a constant that always favors the reaction in one direction.

4. $\Delta S°_{\alpha\rightarrow\beta} = +0.94$ J K^{-1} mol^{-1}, calculated by substituting $\Delta G°$ = −1.43 kJ mol^{-1}, $\Delta H°$ = −1.15 kJ mol^{-1}, and T = 298 K into the equation $\Delta G° = \Delta H° - T\Delta S°$. This value for $\Delta G°_{\alpha\rightarrow\beta}$ was obtained by setting $\Delta G = 0$ in eq 2 and using the reactant:product concentration ratios for the equilibrium at 298 K. The concentrations of A and B are proportional to the mole fractions of each, thus $\Delta G° = -RT\ln(0.64/0.36)$ = (−8.314 J K^{-1} mol^{-1})(298 K) ln(0.64/0.36) = −1.43 kJ mol^{-1}.

Acknowledgments

I thank Boyd Earl and Spencer Steinberg (UNLV) for their helpful comments on the manuscript.

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