

Predictions of Conformational Enthalpy and Heat Capacity from the Zimm–Bragg Theory

Robert A. Kromhout* and Bruno Linder†

Chemical Physics Program, The Florida State University, Tallahassee, Florida 32306-4390

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Statistical mechanical calculations are presented of the conformational enthalpy and heat capacity of a solution of poly- γ -benzyl-L-glutamate using the partition function of Zimm and Bragg for helix–coil transitions. We determine the temperature dependence of the statistical parameter s from optical data and treat the parameter σ as a fitting parameter. We note, however, that consistency with the Zimm–Bragg theory requires that σ not be less than the square of the reciprocal of the number of residues per molecule. We utilize that data given by Ackermann and Rüterjans [Ackermann, Th.; Rüterjans, H. *Ber. Bunsen-Ges. Phys. Chem.* **1964**, 68, 850] and find the same value for σ as they, $\sigma = 2.5 \times 10^{-5}$ for their solution of 0.257 mol of residue per liter in a solvent of dichloroacetic acid and 1,2-dichloroethane. We consider our calculation to be in satisfactory agreement with that of the experiment but point out some problems associated with the determination of the temperature dependence of s .

Introduction

Recently we have published a method¹ for predicting the enthalpy and heat capacity at constant pressure for a simple model of folding of small globular proteins utilizing the statistical mechanical isobaric–isothermal ensemble.^{1,2} In applying this model, we used optical data to determine the equilibrium constant for the conformational change as a function of temperature and from this the enthalpy change because of the change in conformation. Using a simple all-or-none version of our theory together with heat capacity data from temperature regions *outside* the range in which the conformational heat capacity peak occurs, we successfully predicted the heat capacity as a function of temperature within the temperature range of the conformational change, as well as the conformational heat capacity and enthalpy as a function of temperature.

We more recently turned our attention to helix–coil conformational changes. Here, our simple model did not yield satisfactory predictions of enthalpy and heat capacity. We decided to attempt the prediction of the conformational enthalpy and heat capacity from the Zimm–Bragg theory^{3,4} of the helix–coil transition. As before, we decided to focus our attention on a system where optical and heat capacity data were both available. One complication of the Zimm–Bragg theory as compared to our protein-folding theory is that it is expressed in terms of two parameters, s and σ . The first, s , is a statistical weight which is presumably temperature dependent, whereas σ is usually presumed to be temperature independent and can be associated with the average lengths or numbers of residues in helical segments in a large molecule undergoing a helix–coil transformation.⁴

We were unable to find in the literature any quantitative theoretical determinations of conformational enthalpy and heat capacity as functions of temperature derived from the Zimm–Bragg theory. Zimm and Bragg³ set up the partition function

from a simple model of the conformational change and interpreted the meaning of s as an equilibrium constant for incorporating a residue from a nonhelical segment into an already established helical segment; Applequist⁴ derived additional relationships between σ , s , the fraction of helical residues, and the enthalpy change from the addition of a helical residue, all of which we use below. Our results in this paper show that there are some complications in applying the Zimm–Bragg theory to predict enthalpy and heat capacity, some of which are associated with the determination of σ and with the small value of σ which is commonly taken to be the order of 10^{-4} , but the most difficult problem is the determination of the temperature dependence of s .

Method

The Zimm–Bragg partition function is expressed in terms of the statistical weights, s , for each helical residue that is bonded to a helical residue, and a weight σs for each helical residue not so bonded. There is also a parameter μ that specifies the number of residues in each helical turn. As given by Zimm and Bragg³ in their formula (1), the partition functions for $\mu = 1$ is

$$Q = 1 + \sum_{l=1}^{1/2(N-2)} \sigma^l \sum_{k=1}^{N-l-2} s^k \frac{(k-1)!(N-k-2)!}{l!(l-1)!(k-l)!(N-k-l-2)!}$$

for a molecule containing N residues. (For general μ , see Applequist.⁴)

For an α helix, μ is 3 but is often taken to be 1 for simplicity, because for large N the results are nearly the same and the calculations are much simpler.^{3,4}

By the use of the matrix method for evaluating partition functions, Applequist shows that Q can be expressed in terms of the roots, λ , of a secular equation

$$\lambda^{\mu-1}(\lambda-1)(\lambda-s) = \sigma s$$

Thus his eq 19

* To whom correspondence should be addressed: Physics Department, The Florida State University, Tallahassee, FL 32306-4350. Phone: (850) 644-5299. Fax: (850) 644-8630. E-mail: kromhou@mailier.fsu.edu.

† Department of Chemistry.

$$Q = \sigma s \sum_{i=0}^{\mu} \frac{\lambda_i^{N+\mu+1}}{(\lambda_i - 1)[(1+s)\lambda_i^{\mu} - 2s\lambda_i^{\mu-1} + (\mu+1)\sigma s]}$$

For $\mu = 1$ and a large N

$$Q \cong \lambda_0^N$$

where

$$\lambda_0 = \frac{1}{2} \{1 + s + [(1-s)^2 + 4\sigma s]^{1/2}\}$$

(The term in the smaller root, λ_1 , is negligible for $N \geq 500$ for $\sigma \sim 10^{-4}$.)

Use of this simplified expression for Q greatly simplifies the prediction of configurational enthalpy and heat capacity as compared to the exact expression for $\mu = 3$ and an arbitrary N .

Our application of this theory will be to poly- γ -benzyl-L-glutamate where $N = 1007$, so the approximate expression for Q above should be quite adequate.

Appelquist has shown that (1) "the ratio of the populations of two complexions having the same number of hydrogen bonds" (for $\mu = 1$, this is the number of helical residues) "differing by one unbroken sequence of bonds is σ ", (2) "the ratio of the populations of two complexions having the same number of bonded sequences, but differing by one hydrogen bond is s ", (3) the fraction of residues bonded, f , is $f = (1/N) \partial \ln Q / \partial \ln s$, (4) the average number of bonded sequences at the center of the transition ($s = 1$ and $f = 1/2$ in our approximation) is $\langle n_s \rangle = (N/2)\sigma^{1/2}$ if $\sigma \ll 1$, so the average length of a bonded sequence at this point is $\sigma^{-1/2}$, and (5) $\partial f / \partial \ln s|_{s=1} = 1/4\sigma^{-1/2}$.

A key question in our application of this theory is, What is the relationship between s and the temperature T ? This relationship may be introduced^{3,4} by recognizing that s is the equilibrium constant for the addition of one residue to an existing helical chain and write

$$\frac{\partial \ln s}{\partial (1/T)} = -\Delta H^\circ/k$$

where k is Boltzmann's constant and ΔH° is the enthalpy difference between states having $(n+1)$ and states having n helical residues. We will write $h_s = \Delta H^\circ/k$, which has the units of degrees Kelvin. Alternatively we could define an equilibrium constant for the ratio of the population of helical residues to random coil residues

$$K = f/(1-f)$$

Then

$$\frac{\partial \ln K}{\partial (1/T)} = -\Delta H^\circ/k \equiv -h_k$$

There is, of course, no requirement that either h_s or h_k be independent of T or s . Appelquist has shown, however, that at the midpoint of the transition, where $s = 1$

$$h_s(s=1) = h_k(s=1)\sigma^{1/2}$$

or in terms of the fractional helicity, f

$$\partial f / \partial (1/T) = h_s/4\sigma^{1/2}$$

This implies that at this point h_s is of the order of 1% of h_k .

In the spirit of our method in ref 1, we will assume that the statistical parameter s includes the effects of residues interacting with solvent and with each other and that volume changes at constant pressure are accounted for. We treat the Zimm-Bragg partition function as an isothermal-isobaric partition function.

From statistical mechanics, the enthalpy is related to the isothermal-isobaric partition function by

$$\Delta H(T,P) = RT^2 \left(\frac{\partial \ln Q}{\partial T} \right)_P$$

and the heat capacity at constant pressure is given by

$$\Delta C_p(T,P) = \left(\frac{\partial \Delta H(T,P)}{\partial T} \right)_P$$

the Δ implies that this is the conformational part.

In principle, the quantities h_s and/or h_k could be calculated, but because the interactions of residues and solvent molecules at constant pressure are involved, it is desirable to look for nonthermal data from which one or both of these might be estimated. As in the case of protein folding, we again look to optical data for this purpose to test our ability to predict enthalpy and heat capacity for comparison with experiment.

A source of suitable data is found in the paper by Ackerman and Rüterjans,⁵ where optical rotary dispersion data and specific heat data are given for a solution of poly- γ -benzyl-L-glutamate (PBG) in solution in a mixture of dichloroacetic acid and 1,2-dichloroethane. The PBG molecule in this case contains, on the average, 1007 residues; N is thus large enough so that only the largest root of the characteristic equation need be used.

We assumed that at high temperatures all of the residues were found in helical form and normalized the optical data to represent f values between 0 and 1. Then

$$\frac{1}{N} \ln K = \frac{[\ln f - \ln(1-f)]}{N}$$

was plotted versus reciprocal Kelvin temperature as shown in Figure 1. Notice that, for $\ln K$ between plus and minus 1.5×10^{-3} , this graph appears to be quite linear, which implies that h_k is constant. This constant was determined to be 38.4×10^3 K and the midpoint of the transition (where $f = 1/2$ and $\ln K = 0$) was 306.2 K. This would imply, according to Appelquist, that at the midpoint $h_s = 38.4 \times 10^3 \sigma^{1/2}$ K or $h_s = 384$ K if $\sigma = 10^{-4}$.

If $\ln K$ is taken to be linear in $(1/T)$, then $\ln s$ cannot be because of the nonlinear relationship between s and K , $K = s[\delta + s + 2\sigma - 1]/[\delta - s + 2\sigma s + 1]$, with $\delta = [(1-s)^2 + 4\sigma s]^{1/2}$. The resulting relationship between $\ln s$ and $(1/T)$ in a form independent of the value of h_k is shown in Figure 2. The range of reciprocal temperatures for the observed PBG transition is shown on the graph; this range is estimated using the value of h_k taken from the slope in Figure 1. Notice that for values of σ between 2.5×10^{-5} and 1.0×10^{-3} there is significant curvature in the $\ln s$ curves in the temperature range of interest. If one introduces the dependence of s on K explicitly in order to calculate the dependence of $\ln Q$ on temperature, the resulting enthalpy does not asymptotically approach a constant value at high temperatures (Figure 3; nor does the heat capacity display a peak as do the experimental data).

If one replaces the $\ln s$ versus $(1/T)$ relationships shown by Figure 2 by straight lines of slope $\sigma^{1/2}$ (i.e., by straight lines tangent to the Figure 2 curves at $s = 1$), then the curves shown in Figures 4 and 5 result; for σ equal to 2.5×10^{-5} , these are

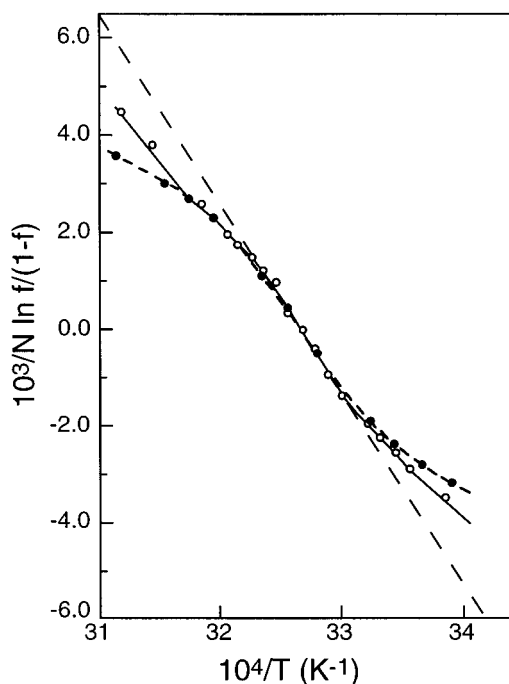


Figure 1. Plot of $(10^3/N) \ln f/(1-f)$ vs the reciprocal of the Kelvin temperature (times 10^4), where f is the fraction of helical residues as measured by optical rotary dispersion from the data in Figure 1 of Ackermann and Rüterjans, $-\circ-$. The dashed line is a straight line of slope 38.4 K, tangent to the experimental curve at $K = 1$ ($f = 1/2$). The curve marked with $-\bullet-$ is calculated for constant $h_s = h_k \sigma^{1/2}$, with $\sigma = 2.5 \times 10^{-5}$ but is essentially the same for $\sigma = 2.0 \times 10^{-4}$. T_m , the temperature at $K = 1$, is 306.2 K; from the central slope, $h_k = 3.84 \times 10^4$ K.

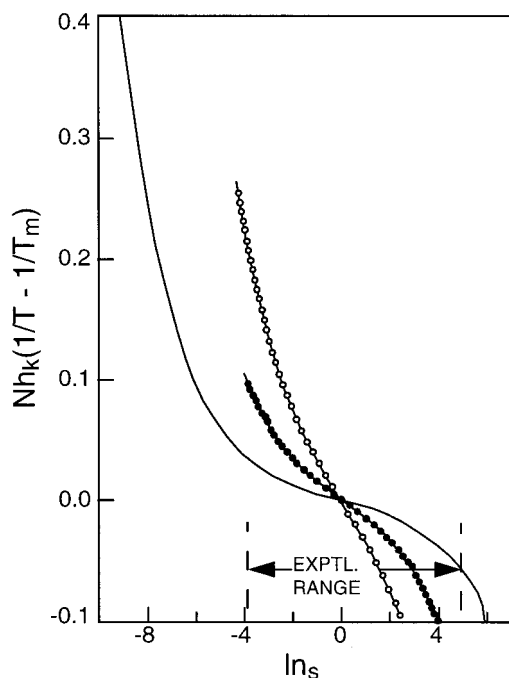


Figure 2. Plots of $\ln s$ vs $N h_k [(1/T) - (1/T_m)]$ calculated for three values of σ from the Zimm–Bragg theory, assuming that h_k is independent of T . Solid curve, $\sigma = 2.5 \times 10^{-5}$; $-\bullet-$, $\sigma = 2.0 \times 10^{-4}$; $-\circ-$, $\sigma = 1.0 \times 10^{-3}$. In each case, the slope at $s = 0$, $T = T_m$, is $\sigma^{1/2}$. The “experimental range” is calculated for $h_k = 3.84 \times 10^4$ K and the observed range of temperatures where the heat capacity differs from zero within experimental error 294.5–315.5 K.

fairly good representations of the experimental data. (The experimental values of the enthalpy were obtained by numerical

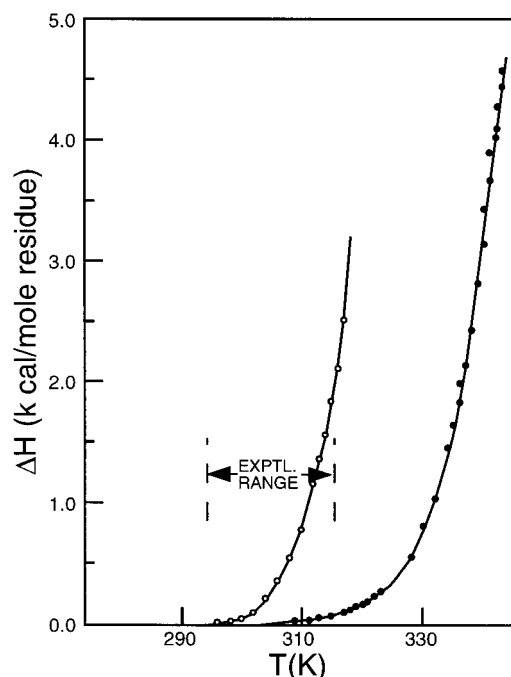


Figure 3. Configurational enthalpy ΔH vs T in Kelvin calculated from the Zimm–Bragg theory, assuming that h_k is independent of temperature and for two values of σ : $-\circ-$, $\Delta H \times 10$, $\sigma = 2.5 \times 10^{-5}$; $-\bullet-$, ΔH , $\sigma = 2.0 \times 10^{-4}$.

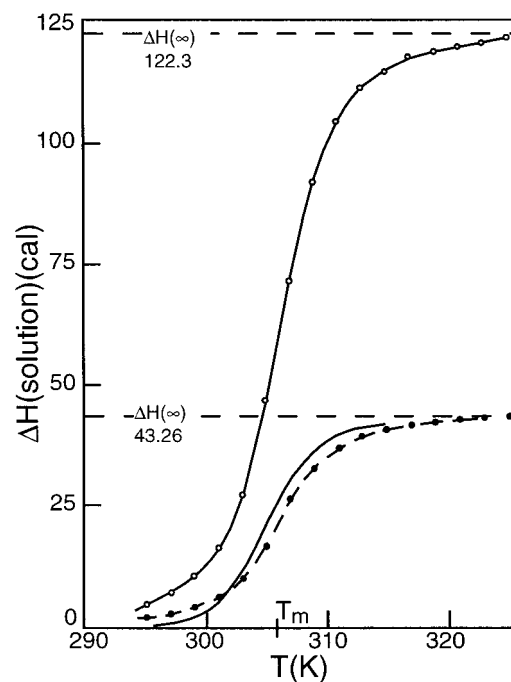


Figure 4. Configurational enthalpy in calories for 438 g of solution, 0.257 mol of PBG residues in 81% dichloroacetic acid and 19% 1,2 dichloroethane by weight: solid curve, experiment; $-\bullet-$, calculated, $\sigma = 2.5 \times 10^{-5}$; $-\circ-$, calculated, $\sigma = 2.0 \times 10^{-4}$. In both calculations, $h_s = 3.84 \times 10^4 \sigma^{1/2}$ is assumed to be independent of temperature. The corresponding asymptotic values of $\Delta H(\infty)$, 43.26 and 122.3 cal, are indicated on the axis and by horizontal dashed lines.

integration of the difference between the experimental C_p curve and a linear background shown by the data of Ackerman and Rüterjans.) The calculated ΔH at 335 K is 43.26 cal for 438 g of solution of 0.257 mol of residue/kg which corresponds to 384 cal/mol of residue. (Our calculated and experimental values are smaller than the experimental value of 430 cal/mol of residue given by Ackerman and Rüterjans.)

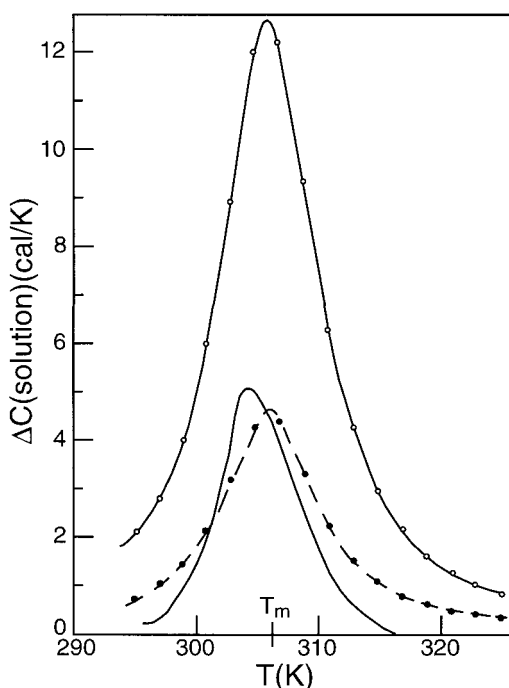


Figure 5. As in Figure 4 but showing heat capacity in calories per Kelvin vs temperature.

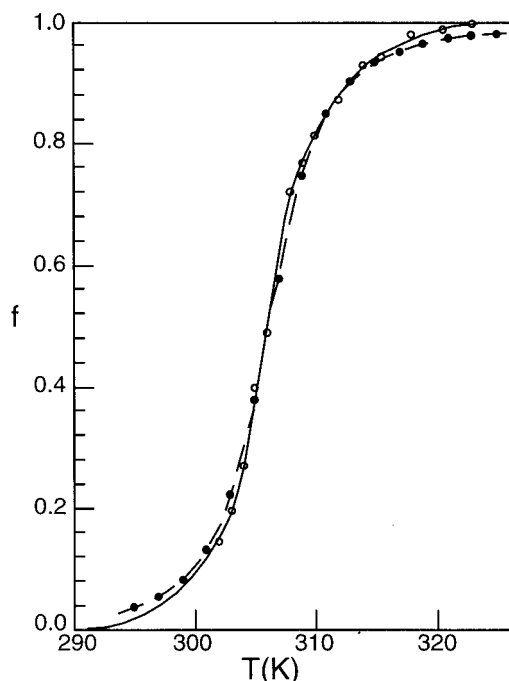


Figure 6. The helicity fraction, f , calculated for $h_s = 3.84 \times 10^4 \sigma^{1/2}$ vs temperature and the experimental f . The calculated f for $\sigma = 2.0 \times 10^{-4}$ cannot be distinguished from that for $\sigma = 2.5 \times 10^{-5}$ on this plot. —●—, theory; —○—, experiment.

It is therefore of some interest to assume that $\ln s$ is linear in $(1/T)$ with the constant slope $38.4\sigma^{1/2} \times 10^3 \text{ K}$ and to calculate $\ln K$ from its dependence on s to see whether a plot of $1/N \ln K$ versus $(1/T)$ from the optical data would be manifestly nonlinear in this case. Figures 1 and 6 show that the deviations from the experimental values appear to be within experimental uncertainties for $\sigma = 2.5 \times 10^{-5}$ and 2.0×10^{-4} . Thus, we take $s = s_0 \exp(-h_s/T)$, where s_0 is chosen so this s is one at T_m .

In the case, where h_s is independent of temperature, we can write the configurational enthalpy as a constant (the asymptotic,

high temperature, configurational enthalpy) times the fractional helicity. Per mole of residues it is

$$\begin{aligned}\Delta H(T) &= RT^2 \frac{\partial \ln \lambda}{\partial T} \\ &= RT^2 \left(\frac{\partial \ln \lambda}{\partial \ln s} \right) \left(\frac{\partial \ln s}{\partial T} \right) \\ &= RT^2 (f) (Nh_s/T^2) \\ &= Rh_k N \sigma^{1/2} f\end{aligned}$$

Applequist has also shown that the rms fluctuation in the number of helical residues is $N^{1/2}/2\sigma^{1/4}$ or 224 at the midpoint, which per helical residue is $(N\sigma^{1/2})^{-1/2}$ or 0.199 for $\sigma = 2.5 \times 10^{-5}$. If h_s is independent of temperature, we have shown above that $\Delta H(T)$ is simply proportional to the number of helical residues. Thus, the relative fluctuation in the enthalpy is (Hill² p 103)

$$(H - \bar{H})^2 / \bar{H}^2 = kT^2 \Delta C_p / (\Delta H)^2$$

Using this and the value of the rms fluctuation in the number of helical residues at T_m , it is easy to derive the relation between the maximum ΔC_p , the asymptotic ΔH , and σ given by Ackermann and Rüterjans as their eq 17 (Ackermann and Rüterjans⁵ p 885):

$$\Delta C_{p,\text{max}} = \frac{1}{4\sigma^{1/2}} \frac{[\Delta H(\infty)]^2}{RT^2}$$

When σ is a very small number, the average length of an uninterrupted sequence is large (for $\sigma = 2.5 \times 10^{-5}$, it is 200, and for $\sigma = 2 \times 10^{-4}$, it is 72).

If σ were 10^{-6} , the behavior would be “all-or-none” because $\sigma^{-1/2}$ would be 1000, whereas N is 1007; the rms fluctuation would be 0.993 per residue, and the asymptotic enthalpy, $\Delta H(\infty)$, would be Rh_k or 76.9 cal per mole of residue. Thus σ can be determined by the experimental ΔH (or $\Delta C_{p,\text{max}}$), h_k , and N . The minimum value of σ is $1/N^2$.

Our efforts to predict the conformational enthalpy and specific heat utilizing optical data and the Zimm–Bragg theory are satisfactory. The most serious problem is the lack of access to the temperature dependence of the statistical factor s except in the immediate neighborhood of the midpoint, T_m , where the optical data gives the standard state enthalpy change for the transfer of one residue from random coil bonding to helical in terms of the temperature dependence of the optically determined helicity; unfortunately, from our point of view, this relationship also involves the unknown σ . Our attempt to obtain σ by assuming the standard state enthalpy change so obtained to be temperature independent and fitting the largest values of ΔC_p and ΔH to the experimental values yielded for σ the value suggested by Ackermann and Rüterjans, 2.5×10^{-5} , significantly smaller than the value (2×10^{-4}) usually assumed. Use of $\sigma = 2 \times 10^{-4}$ would yield a peak value of ΔC_p about 2.4 times the experimental value and a maximum ΔH value about 2.8 times too large.

We note that our predicted ΔC_p curves in Figure 5 all have greater half-widths as well as nonnegligible values where the experimental curve is indistinguishable from the background. We also note that, according to Ackermann and Rüterjans, the value of σ changes with concentration in this system, approaching 0.9×10^{-4} at infinite dilution.

We have calculated f and $\ln K$ for h_s independent of temperature and for both $\sigma = 2.5 \times 10^{-5}$ and 2.0×10^{-4} . The

$\ln K$ results appear in Figure 1, whereas the f values appear in Figure 6, along with the values obtained from the optical data. For $h_s = 3.84 \times 10^4 K \sigma^{1/2}$, the difference between the values of $1/N \ln K$ and of f for these two values of σ is negligible; for smaller values of h_s , the curves can be distinguished. The fact that the theoretical curve for $-\ln K$ does not fit well the data for large and small K is probably not significant because the optical data has large relative errors for f or $(1 - f)$ small. It is clear that the $\ln K$ and f curves are too insensitive to σ to provide effective fitting opportunities, whereas ΔH and ΔC_p can be used very effectively for this purpose.

As opposed to predicting configurational enthalpy and heat capacity as a function of temperature, one could use $h_s(T)$ and σ as parameters to be determined by least-squares fit to the experimental C_p and optical data and include, as well, the

possibility that the maximum fraction of helicity is some f_m less than one.⁶ Because good theoretical calculations of h_s do not appear feasible, one must question the value of such an exercise. Should a constant value of h_s not provide a good fit to the data, however, the dependence of s on T would otherwise be difficult to deduce.

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