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Possible origin of differences between van't Hoff and calorimetric enthalpy estimates

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Abstract

Monte Carlo simulations were used to reveal a subtle, hidden contribution to van't Hoff enthalpy estimates from small heat capacity changes. In simulated van't Hoff plots of ln K versus $1/T$, small heat capacity changes ($-200 \leq \Delta C_p \leq +200$ cal mol^{-1} K^{-1}), for which curvature could not be discerned within the noise of the data, were found to systematically bias the slope, leading to apparently erroneous enthalpy estimates. Nonlinear least squares analysis of the simulated van't Hoff plots further revealed that it is difficult to extract statistically reliable ΔC_p values from data with even modest noise levels. Estimates of ΔC_p and ΔH_{vH} were found to be highly correlated, indicating an ill-posed nonlinear fitting problem. Nonlinear fits were found in many cases to be statistically no better than simpler linear fits. These simulations show, however, that if an independent calorimetric enthalpy estimate is available, apparent discrepancies between that value and estimates derived from van't Hoff plots may be used to infer the existence of a small heat capacity change and its sign. By this procedure, apparent differences between van't Hoff and calorimetric enthalpy estimates may be interpreted and reconciled. An important conclusion from these studies is that such differences most likely result for statistical reasons, rather than from underlying physical causes.

Keywords: Enthalpy; Calorimetry; Monte Carlo simulation; Parameter estimation; van't Hoff plots

1. Introduction

Enthalpy estimates for chemical and biochemical reactions are often made indirectly from studies of the temperature dependence of equilibrium constants. The well-known van't Hoff equation

$$\{\mathrm{d}\ln K/\mathrm{d}T\} = \Delta H_{\mathrm{vh}}/RT^2$$

or its alternate form

$$\{\mathrm{d}\ln K/\mathrm{d}T^{-1}\} = -\Delta H_{\mathrm{vh}}/R$$

may be used to obtain the enthalpy (ΔH_{vH}) from such data [1]. In these equations, K is the equilibrium constant, T the absolute temperature, and R the gas constant. In the absences of heat capacity changes, van't Hoff plots of ln K versus $1/T$ should be linear, with a slope equal to $-\Delta H_{\mathrm{vH}}/R$. If the enthalpy varies with temperature, the heat capacity change, $\Delta C_p = (\delta \Delta H/\delta T)_p$, is nonzero, leading to possible curvature in van't Hoff plots. For analysis of the complete van't Hoff curve in such cases, more

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complicated models must then be used in attempts to extract reliable estimates of ΔH_0 and ΔC_p from experimental data [2]. If van't Hoff plots are curved, the tangent to the curve at any point along the curve is ΔH at that temperature.

When values of van't Hoff enthalpy estimates for a particular reaction are compared to enthalpy estimates for the same reaction obtained directly by calorimetric methods, there is often substantial disagreement between the two estimates [2]. Results from this laboratory may be used as an example. The enthalpy for the interaction of the anticancer drug daunomycin with DNA has been estimated from both the temperature dependence of its binding constant (over the range of 12–50°C) and directly by direct microtitration calorimetry at 25°C (D. Suh and J.B. Chaires, unpublished data). The van't Hoff enthalpy estimate was found to be -13.3 (± 0.6) kcal mol^{-1} (1 kcal = 4.184 kJ), while the calorimetric enthalpy was found to be $-10.4(\pm 1.0)$ kcal mol^{-1}. The values differ by 28%, a difference that is, given the error in each measurement, statistically significant. Sturtevant and co-workers [3] have recently reported differences in van't Hoff and calorimetric enthalpy estimates for several diverse systems, ranging from the interaction of 2′-CMP with RNase A to the formation of a cyclohexanol–cyclodextrin inclusion complex. These differences were interpreted to be physically significant, and to reflect the underlying complexities of the reactions studied. It is possible, however, that differences in calorimetric and van't Hoff enthalpy values arise from an inappropriate choice for the fitting function used to analyze van't Hoff plots [2], or from other difficulties in parameter estimation in the least squares fitting procedures used.

A more insidious reason for differences between van't Hoff and calorimetric enthalpy values was recently suggested by Weber [4], who discussed the limited reliability of van't Hoff plots in the determination of enthalpy values for the association of protein subunits. Weber argued that if ΔH and ΔS are temperature dependent, in violation of the usual assumption made in deriving the van't Hoff equation, enthalpy values obtained from van't Hoff plots may systematically differ from the true enthalpy values. The magnitude of the difference may be substantial. Vociferous rebuttals to the article by Weber followed [5,6], in which it was argued that the basic premise of his argument was incorrect, and that his subsequent development was therefore fatally flawed.

Whether or not differences in van't Hoff enthalpy estimates arise from real underlying physical reasons, or from more mundane difficulties in the proper analysis of van't Hoff data is a problem that demands further attention. Reported here are the results of Monte Carlo simulations that explore the basis of the apparent discrepancy between van't Hoff and calorimetric enthalpy estimates. The results of these simulations lead to the disturbing conclusion that small values of ΔC_p can make a hidden contribution to data cast into the form of a van't Hoff plot. Small ΔC_p values are shown to bias the slope of van't Hoff plots without producing curvature in the plot that is discernible within realistic noise levels in the data, leading to apparently erroneous enthalpy values when such data are compared to the true enthalpy value or to a calorimetrically determined value obtained at a single temperature. On the positive side, however, the simulations show that if a calorimetrically obtained enthalpy estimate is available, the discrepancy between its value and one obtained by van't Hoff analysis might be used to infer the existence of a ΔC_p small in magnitude. Further, the simulations show that the apparent differences between the van't Hoff and calorimetric enthalpy estimates are more illusory than real, and arise from differences in the temperatures to which the two enthalpy values refer, which results in unequal ΔH values because of nonzero ΔC_p values.

2. Methods

Monte Carlo simulation techniques [7,8] were implemented in an algorithm written using MATHCAD PLUS 5.0 (MathSoft, Inc., Cambridge, MA). The overall approach was as follows. Perfect data were simulated using an appropriate function. The data were then perturbed by the addition of pseudo-random noise. The perturbed data were then fit by least squares methods to selected models. This process was repeated numerous times, each time adding a different set of noise to the perfect data. The best fit parameters were accumulated and averaged.

Perfect data were generated using the following integrated form of the van't Hoff equation [9]:

$$\ln K = \ln K_r + \left[\left(-\Delta H_r + \Delta C_p T_r\right)/R\right)\left(T^{-1} - T_r^{-1}\right)\right] + \left(\Delta C_p/R\right)\ln(T/T_r) \qquad (1)$$

where R is the gas constant, T the absolute temperature, K the equilibrium constant, ΔH the enthalpy change, and ΔC_p the heat capacity change. The subscript r refers to values at a fixed selected reference temperature, T_r. For selected values of $\ln K_r$, ΔH_r, ΔC_p, and T_r, 10 equally spaced $\ln K$ values were calculated over a temperature range of 283.15–323.15 K. Such a temperature range is typical of that usually found in studies of biochemical systems. The perfect data were then perturbed by adding pseudo-random noise at a selected level. The apparent van't Hoff enthalpy value (ΔH_{vH}) was then obtained from the perturbed data by a linear least squares fit. The process was repeated 1000 to 5000 times to accumulate a distribution of ΔH_{vH} values from which average values were computed. At each repetition, a different set of random noise was added to the perfect data, but the magnitude of the added noise was constant. In additional studies, perturbed data were fit to both a straight line and to Eq. (1) using a nonlinear least squares method based on the Marquardt–Levenberg algorithm [10]. The goodness of fit of the linear and nonlinear fits was compared by examination of the sum of the square of the residuals. Analytical derivatives were obtained using the software DERIVE, v. 2.5, (Soft Warehouse, Inc., Honolulu) [11].

3. Results and discussion

Fig. 1 shows simulated van't Hoff plots that exemplify the subtle bias introduced by small heat capacity changes. Fig. 1(A) shows perfect data calculated using Eq. (1), unperturbed by any noise. The small ΔC_p value (-200 cal mol^{-1} K^{-1}) introduces only slight curvature over the temperature range studied. A linear fit yields a slope of 6877, from which $\Delta H_{vH} = -13.6$ kcal mol^{-1} may be calculated, a value significantly different from the $\Delta H = -10.4$ kcal mol^{-1} (at 20°C) used to simulate the data. The linear correlation coefficient of the fit is 0.995, a value that would usually indicate an acceptable fit, even though in this case a linear fit represents a clearly incorrect model. The standard deviation in the fit is 0.11. Only upon inspection of the residuals (Fig. 1(A), inset) does the systematic deviation from the incorrect linear fit become evident. When pseudo-random noise (Fig. 1(B)) is added to the data, the systematic deviations from linearity become lost in the noise (Fig. 1(B), inset). The noise level introduced in this example is modest, equal to an average deviation in ln K of only 2%. The slope of the noise perturbed data in Fig. 1(B) is 6412, leading to $\Delta H_{vH} = -12.7$ kcal mol^{-1}, and the linear correlation coefficient is 0.976. The standard deviation of the fit is 0.27. In the absence of other information, there would be no reason not to judge the linear fit in Fig. 1(B) acceptable. Any curvature that should result from the small ΔC_p is lost within the noise, but the bias in the slope remains.

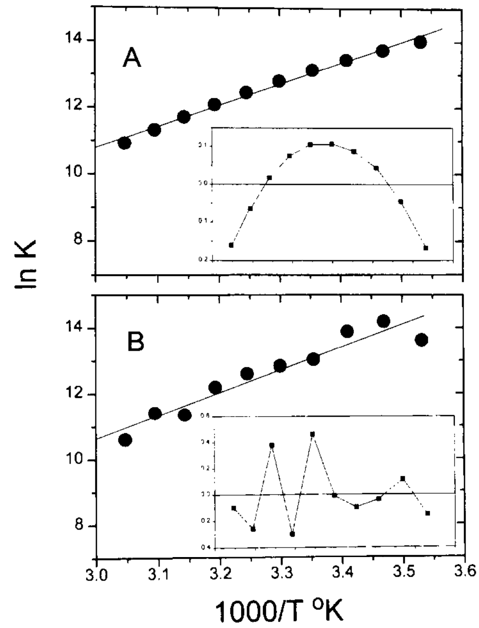

Fig. 1. Simulated van't Hoff plots. Values of ln K were calculated over the range 283.15–323.25 K using Eq. (1), with the parameters $\ln K_r = 13.46$, $T_r = 293.15$, $\Delta H_r = -11.0$ kcal mol^{-1}, and $\Delta C_p = -200$ cal mol^{-1} K^{-1}. (A) Unperturbed data: the line is the linear least squares fit to the data. (B) One example of noise perturbed data: the line is the linear least squares fit.

The reason for the increased slope may be seen from an examination of the derivative of Eq. (1). The derivative of Eq. (1) with respect to $1/T$ is

$$(\mathrm{d}\ln K/\mathrm{d}T^{-1}) = -\Delta H/R$$
$$= (\Delta H_{\mathrm{r}} + \Delta C_p(T - T_{\mathrm{r}}))/R$$

Upon cancellation of R, the result is that

$$\Delta H = \Delta H_r + \Delta C_p(T - T_r)$$

The apparent slope at each point along the curve varies if $\Delta C_p \neq 0$. The slopes in Fig. 1 results, evidently, from a T value corresponding to the mean value of the 10 temperatures used in the simulation, and ΔH from the van't Hoff plot would refer to that temperature. The subtle error results when comparing such a value with ΔH obtained at a single reference temperature by calorimetry. Because curvature in the van't Hoff plot due to small ΔC_p values is lost within even modest amounts of noise, one would not expect a temperature dependence of ΔH, nor be likely to interpret the apparently linear van't Hoff plot as reflecting anything other than a temperature-dependent enthalpy.

Monte Carlo methods were used to explore the effects of a range of ΔC_p values and a range of added noise levels. In these studies, 1000 to 5000 cases were simulated, and the apparent ΔH_{vH} values accumulated and averaged. Fig. 2 shows the results of these simulations. These data show that ΔC_p introduces a systematic bias into the slope of van't Hoff plots, resulting in computed ΔH_{vH} values that deviate from the true value used to generate the data. In all of these cases, as in the single example shown above, the linear correlation coefficients were high, and there would be no reason to suspect that anything other that a linear fit to the data was needed or appropriate. A range in added noise corresponding to an average deviation in ln K of 1 to 4% was explored. The average ΔH_{vH} obtained at a given ΔC_p value was found to be independent of the magnitude of the added noise, but increased noise resulted in increased uncertainty in the mean value, as expected. The results of Fig. 2 lead to the disturbing conclusion that small ΔC_p values can bias the slope of van't Hoff plots, and lead to apparent systematic errors in ΔH_{vH} values

It is possible that these apparently erroneous enthalpy estimates arise simply from the failure to fit the data to the appropriate nonlinear model, Eq. (1). Again, it must be stressed that in the noise-perturbed data, curvature in the data that would lead one to consider more complicated nonlinear models is not readily apparent. Nonetheless, further simulations were conducted in which linear fits were compared with nonlinear fits using Eq. (1). In these simulations, data were generated using Eq. (1) and the following parameters: ln $K_{\mathrm{r}} = 13.46$; $T_{\mathrm{r}} = 293.15$; $\Delta H_{\mathrm{r}} = -10.4$ kcal mol^{-1}; $\Delta C_p = -171$ cal mol^{-1} K^{-1}. Pseudo-random noise was added corresponding to an average deviation of 2% in ln k. A total of 117 cases were generated, and each data set was fit to both a simple straight line and to Eq. (1) using a nonlinear least squares fitting method. From the simple linear fits, an average value of $\Delta H_{\mathrm{vH}} = -12.4 \pm 1.4$ kcal mol^{-1} was obtained, a value 20% higher than the true value used to generate the data. Fig. 3 shows the results of nonlinear fits to the data. Average fitted value of $\Delta H = -10.4 \pm 2.4$ kcal mol^{-1} and $\Delta C_p = -163 \pm 163$ cal mol^{-1} K^{-1} were obtained. While at first glance these values appear to

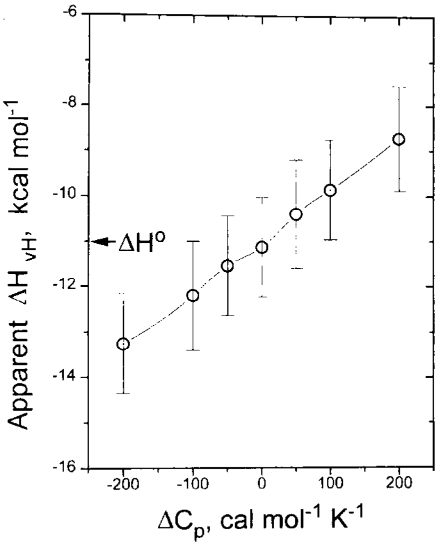

Fig. 2. Results from Monte Carlo simulations. Eq. (1) was used to calculate ln K values over the range 283.15–323.25 K using with the parameters ln $K_{\mathrm{r}} = 13.46$, $T_{\mathrm{r}} = 293.15$, $\Delta H_{\mathrm{r}} = -11.0$, and ΔC_p as indicated. Pseudo-random noise corresponding to a 2% average deviation in ln K was added, and the perturbed data fit by linear least squares methods to estimate ΔH_{vH}. Average values and standard errors were calculated from 1000 to 5000 separate cases. The "true" enthalpy, ΔH^0, used to generated the perfect data is indicated.

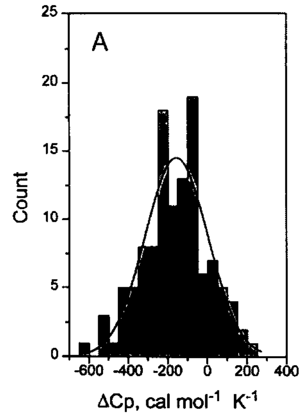

Fig. 3. Distributions of fitted ΔC_p and ΔH^0 values from Monte Carlo simulations with nonlinear least squares fitting of the data. Parameters used to generate data from Eq. (1) were: ln $K_r = 13.46$; $T_r = 293.15$; $\Delta H_r = -10.4$ kcal mol^{-1}; $\Delta C_p = -171$ cal mol^{-1} K^{-1}. A total of 117 cases was analyzed in the simulation.

suggest that the nonlinear fit returns values that match those used to generate the data more closely than does the simpler linear fit, there are clear signs of difficulties. The error in ΔC_p is as large as the parameter itself, indicating that ΔC_p is essentially indeterminate. Fig. 4 shows, in addition, that estimates of ΔC_p and ΔH are highly correlated. Such a correlation occurs with an ill-posed fitting problem [12], and indicates that too many parameters have been included in the model used to analyze the data. Finally, one can examine the goodness of the fits to the linear and nonlinear models (Fig. 5). Fig. 5 shows that in 57 of the 117 cases examined, the linear fit is statistically as good as or better than the nonlinear fit, and that there would be no basis for choosing the more complicated model including the additional parameter. These studies show that small heat capacity changes present a substantial quandary. Small ΔC_p values can bias the slopes of van't Hoff plots and lead to erroneous enthalpy estimates when such data is analyzed by linear least squares fits, as is usually done. On the other hand, fits of data containing small ΔC_p contributions with even modest levels of noise present an ill-posed fitting problem. In such data, both ΔH and ΔC_p cannot be reliably extracted from the data by nonlinear fitting to Eq. (1).

In order to explore an alternate analysis strategy, simulations were conducted with data generated us-

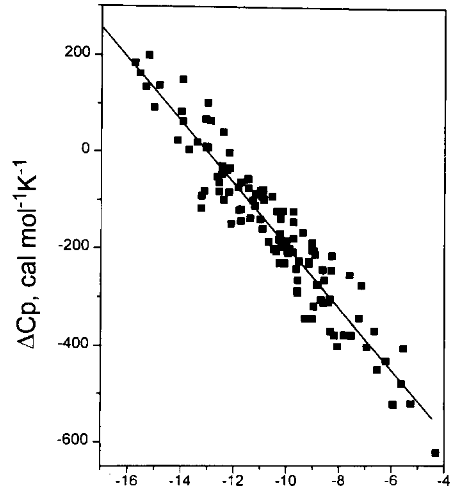

Fig. 4. Correlation of fitted ΔC_p and ΔH^0 values from Monte Carlo simulations. The line is the result of a linear least squares fit, with a correlation coefficient of 0.952.

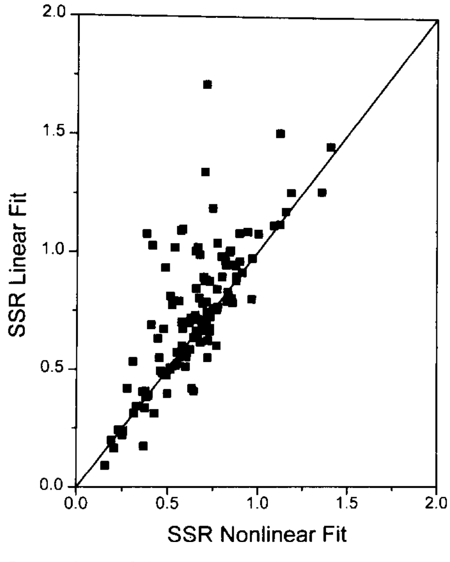

Fig. 5. Comparison of the goodness of linear and nonlinear fits in Monte Carlo simulations. The sums of the square of residuals (SSRs) from linear and nonlinear fits of the same data set are plotted. The diagonal line has a slope of 1. Points above the line indicate a worse statistical fit by the linear model compared to the nonlinear model that includes a contribution from ΔC_p. Points below the line indicate a better fit by the simpler linear model.

ing the same parameters as described in the previous case. Both linear and nonlinear fits were done, but in nonlinear fits the enthalpy was constrained to the "true" value used to generated the data. The nonlinear fits in this case optimized for a single parameter, ΔC_p. This simulation would correspond to a situation in which a reliable independent enthalpy estimate was available at a single temperature, as obtained, for example, by isothermal titration calorimetry. A total of 128 cases were used in the simulation. From the distribution of the fitted values of ΔC_p (data not shown), an average value of -164 ± 46 cal mol^{-1} K^{-1} was determined. The error estimate in ΔC_p is greatly improved over the previous simulation in which two parameters were optimized. In this simulation, the linear fit was as good as or better than nonlinear fits in 63 of the 128 cases, again suggesting that the preference for the nonlinear model is marginal. The simulation suggests, however, that if a reliable, independent calorimetric enthalpy estimate at a fixed temperature is available, data in a van't Hoff plot can provide important additional information. A van't Hoff enthalpy that differs from the calorimetrically determined value might be interpreted as resulting from a small ΔC_p contribution, even though curvature may not be readily apparent in the van't Hoff plot. The nature of the deviation, larger or smaller than the calorimetric estimate, provides a qualitative indication of the sign of ΔC_p. Further, by constraining the enthalpy to the calorimetrically determined value, Eq. (1) might be used in nonlinear fitting of the data to obtain a reasonably reliable estimate of magnitude of ΔC_p.

Additional simulations were done to attempt to evaluate the magnitude of ΔC_p at which nonlinearity in van't Hoff plots is unambiguously evident within the noise of the data. Fig. 6 shows selected results from the simulations. Fig. 6 shows that for ΔC_p

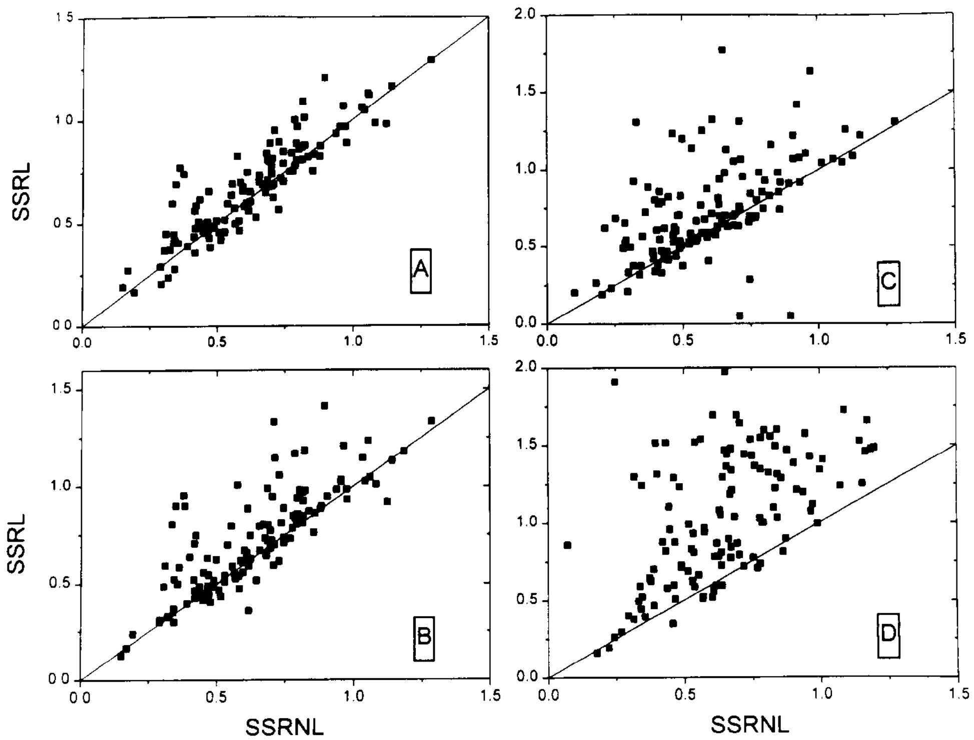

Fig. 6. Comparison of goodness of fit for linear and nonlinear fits to van't Hoff data for data simulated with different ΔC_p values. The sums of the square of residuals obtained from linear (SSRL) or nonlinear (SSRNL) fits obtained from Monte Carlo simulations are plotted, as described in Fig. 5. Parameter values for the simulations were the same as used in Fig. 4Fig. 5, except ΔC_p was varied: (A) $\Delta C_p = 0$; (B) $\Delta C_p = -100$; (C) $\Delta C_p = -200$; (D) $\Delta C_p = -400$ cal mol^{-1} K^{-1}.

values from 0 to -100, the ability to distinguish linear and nonlinear van't Hoff plots is marginal, with linear fits often obtained that are as good as or better than nonlinear fits. For ΔC_p values equal to or greater than -200, the ability to detect nonlinearity becomes more likely, with nonlinear fits statistically better than linear ones in the majority of cases. As shown in Fig. 6(D), for $\Delta C_p = -400$, in only a very few cases is the linear fit marginally better than the nonlinear one. The conclusion from these simulations is that small heat capacity changes, $0 < \Delta C_p < -200$, present the most difficult fitting problem. For values of $|\Delta C_p| > 200$, nonlinearity should be evident, and fits to appropriate nonlinear models ought to yield meaningful parameter estimates provided an adequate range in temperature is covered.

The findings of these simulations should serve to help understand and reconcile the discrepancies often encountered when comparing calorimetric and van't Hoff enthalpy estimates. It is, of course, best to determine both ΔH and ΔC_p values by calorimetry. This may not always be possible because of sample requirements or solubility problems, in which case there are few options for determining enthalpy values other than van't Hoff analysis. Given accurate estimates of equilibrium constants and analysis by appropriate fitting functions, calorimetric and van't Hoff enthalpy estimates should agree. The studies reported here show why they may not, and how such a discrepancy might be turned to an advantage to provide a diagnostic for a hidden contribution from small heat capacity change. In order to illustrate the applicability of the approach presented here, two cases from the published literature will be examined in detail.

Koblan and Ackers [13] published a van't Hoff enthalpy estimate for the interaction of cI repressor with its O_R1 binding site using data obtained by quantitative footprinting. A value of $\Delta_{vH} = -23.3$ kcal mol^{-1} was obtained from van't Hoff plots constructed from five points covering a range of 5–37°C. The plot was linear within the error of the data. Subsequently, an isothermal titration calorimetric study from the same laboratory reported values of $\Delta H_{cal} = -28.7$ (± 1.7) kcal mol^{-1} at 27°C and $\Delta C_p = -550$ cal mol^{-1} deg^{-1} for the same interaction [14]. These values for ΔH_{cal} and ΔC_p were used in the Monte Carlo procedure described here to simulate van't Hoff plots over the range of 5–37°C to obtain a predicted enthalpy of -23.9 (± 1.6) kcal mol^{-1}, a value in excellent agreement with that initially reported by Koblan and Ackers [13]. In the Monte Carlo simulations, nothing other than linear fits to the data over the rather narrow temperature range available was warranted. This exercise reinforces the conclusion that small heat capacity changes can bias the slope of van't Hoff plots without introducing curvature detectable within the error of the data, and further shows how van't Hoff and calorimetric enthalpy estimates that are at first glance disparate might be reconciled. It must be noted that the authors of the calorimetric study [14] correctly demonstrated that the magnitude of the heat capacity change obtained would produce deviations from linearity in van't Hoff plots over the range of 5–37°C that would not be detectable within the noise of the data. The Monte Carlo method described here provides an alternate verification of their conclusion.

Sturtevant and co-workers [3] reported what they believed to be significant differences between calorimetric and van't Hoff enthalpy estimates. In their procedure, isothermal calorimetric titrations were conducted at several temperatures. A calorimetric enthalpy was determined directly at each temperature, and heat capacity changes were determined from the temperature dependency of these enthalpies. Binding constants were obtained at each temperature from the analysis of the primary calorimetric titration data, which allowed Sturtevant and co-workers to construct van't Hoff plots which were then fit to an equation analogous to Eq. (1), yielding separate estimates of ΔH and ΔC_p. From these latter quantities, "van't Hoff" enthalpies were calculated at each temperature used experimentally, and compared to the calorimetrically obtained values. Apparently, substantial differences were reported. Depending on the type of interaction studied, van't Hoff enthalpy estimates were either systematically lower or greater in magnitude when compared to the calorimetric values, with values of the ratio $\Delta H_{vH}/\Delta H_{cal}$ ranging from 0.6 to 1.6 for the different systems. The possible difficulty in this approach lies in the ability to extract reliable ΔH and ΔC_p estimates by nonlinear least squares fitting procedures. One case from [3] will be considered and reevaluated by the Monte Carlo procedure described here, using the data presented in Table 1 of the reference. For the binding of 2′-CMP to RNase A at pH 5.5 and 25°C, Sturtevant and co-workers obtained a calorimetric enthalpy of -10.6 kcal mol^{-1} and $\Delta C_p = -198$ cal mol^{-1} deg^{-1}. From nonlinear fits of van't Hoff plots, values of $\Delta H = -8.97$ kcal mol^{-1} (25°C) and $\Delta C_p = -287$ cal mol^{-1} deg^{-1} were obtained. The issue is whether these differences are statistically significant. First, these data were reanalyzed by nonlinear least squares fitting, but using a different method [12] for the evaluation of the error in parameter estimates. The reanalysis yielded best fit parameters of $\Delta H = -9.02$ kcal mol^{-1} (25°C) and $\Delta C_p = -250$ cal mol^{-1} deg^{-1}, in excellent agreement with the published values. However, the error bounds at the 65% confidence level were $< -10.1; -7.9 >$ for ΔH and $< -437; -60 >$ for ΔC_p. The ratio $\Delta H_{\text{vH}}/\Delta H_{\text{cal}}$ is, with appropriate propagation of error, computed to be $1.17_6 \pm 0.16$. These more realistic error estimates suggest that there is, in fact, little or no difference in the van't Hoff and calorimetric parameter estimates. The Monte Carlo method offers an alternate method for evaluating the error in parameter estimates [7,8]. Monte Carlo simulations were conducted using the calorimetrically determined values for the enthalpy and heat capacity change, generating data to cover the temperature range of 15–40°C that was used in the experiment. The distribution of ΔH and ΔC_p values obtained from 144 simulations are shown in Fig. 7. The averaged values obtained from these distributions were $\Delta H = -10.7$ (± 0.75) kcal mol^{-1} (25°C) and $\Delta C_p = -180$ (± 87) cal mol^{-1} deg^{-1}. The noise added in the simulation corresponded to an average deviation of 0.5% in ln K values, an error corresponding closely to that reported in the experiment. The results of this Monte Carlo simulation show that the error in parameter estimation is likely to be far greater than reported by Sturtevant and co-workers in their analysis of van't Hoff plots, and that if a larger number of plots were analyzed the apparent discrepancy between van't Hoff and calorimetric enthalpies would likely disappear. The apparent differ-

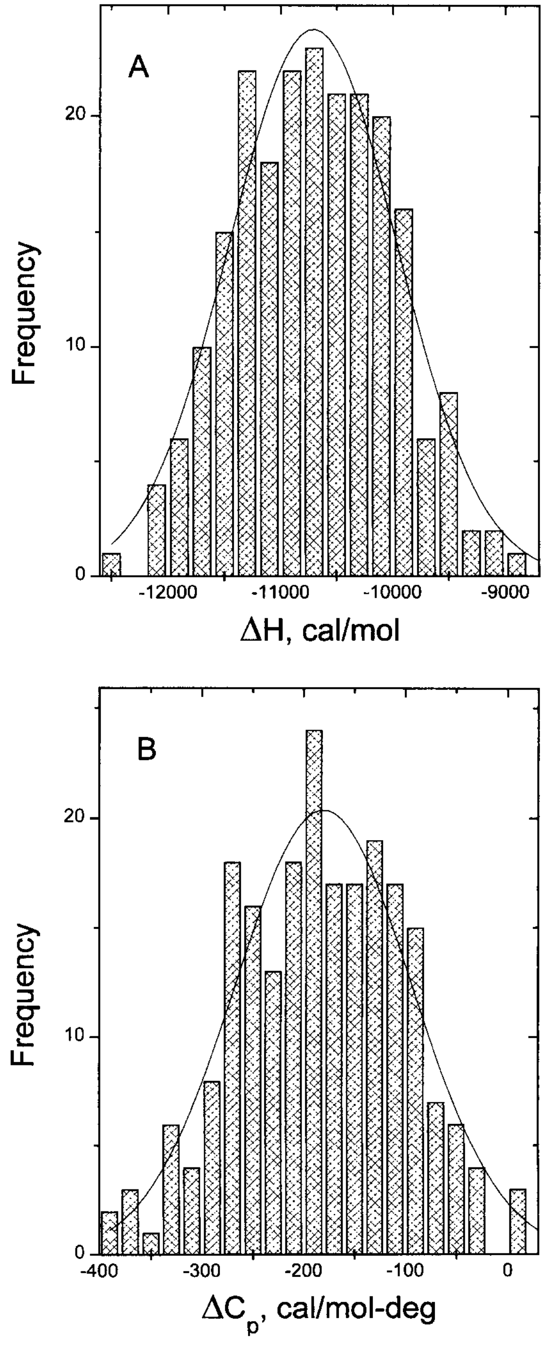

Fig. 7. Results of Monte Carlo simulations of van't Hoff data corresponding to the results described by Sturtevant and co-workers [3] for the binding of 5′-CMP to RNase A. (A) Distribution of fitted enthalpy values. The mean value is -10.7 kcal mol^{-1}, with a standard deviation of ± 0.75. (B) Distribution of fitted ΔC_p values. The mean is -180 cal mol^{-1} deg^{-1}, with a standard deviation of ± 87.

ences in van't Hoff and calorimetric enthalpy estimates reported by Sturtevant and co-workers [3] are most likely a result of the difficulty in the determination of statistically reliable ΔH and ΔC_p values from van't Hoff plots that cover a narrow temperature range, and are unlikely to indicate physically significant differences in the two quantities.

4. Summary and conclusions

The key points that emerge from these simulations are:

1. Small heat capacity changes can bias the slope of van't Hoff plots without producing curvature that is visible within the noise level of typical data.
2. Analysis of van't Hoff data containing small ΔC_p contributions presents an ill-posed and difficult fitting problem. Estimates of ΔH and ΔC_p are highly correlated, and neither parameter can be reliably estimated unless one can be constrained.
3. If a reliable independent estimate of ΔH is available, ΔC_p may be reliably estimated from van't Hoff plots using nonlinear least squares analysis.
4. Apparent discrepancies between van't Hoff and calorimetric enthalpy estimates may be more illusory than real if small heat capacity changes are present in the system under study. The Monte Carlo approach described here can be used to interpret and reconcile such apparent differences.

Acknowledgements

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