# The Observed Change in Heat Capacity Accompanying the Thermal Unfolding of Proteins Depends on the Composition of the Solution and on the Method Employed To Change the Temperature of Unfolding†

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*Received September 14, 1995; Revised Manuscript Received December 4, 1995*⊗

ABSTRACT: The apparent change in heat capacity, $\Delta C_p$, accompanying the thermally induced unfolding of lysozyme and of ribonuclease A was determined by means of differential scanning calorimetry in dilute aqueous buffer containing one of the following added solutes: 0.5 M or 1.0 M sucrose, 1.0 M glycine, 0.5 M, 1.0 M, or 2.0 M guanidinium chloride, 10% glycerol, or 0.5 M NaCl over a pH range. In each system the temperature of half-completion, $t_{1/2}$, of the unfolding transition was varied by varying the pH. The resulting enthalpies of denaturation were linearly dependent on $t_{1/2}$ for each solvent system. The resulting values of $\Delta C_p$ for each protein showed variations of almost 2-fold. Such large variations in the sensitivity of the proteins to temperature changes are not readily interpreted.

The change in heat capacity, $\Delta C_p$, is one of the most important thermodynamic properties characterizing a chemical process, primarily because it essentially controls the variation with temperature of the enthalpy, entropy, and free energy changes accompanying that process. In this paper we are concerned with the heat capacity change accompanying the thermally induced unfolding of a protein and the procedures available for its determination. We will restrict our attention to cases where only two macroscopic states are significantly populated during the unfolding process, and the process is essentially reversible so that equilibrium thermodynamics is applicable.

Ideally the heat capacity change accompanying the unfolding of a protein would be evaluated by determining the difference in the heat capacity of the unfolded protein and that of the folded protein under identical conditions of temperature and solution composition, but this is obviously impossible. Therefore, indirect procedures have to be employed, and these lead to uncertainties of interpretation.

A frequently employed approach for determining the $\Delta C_p$ of protein unfolding is by means of differential scanning calorimetry (DSC). The usual procedure is to vary the temperature of unfolding by varying the pH, and thus to determine the dependence of the calorimetrically observed enthalpy of unfolding, $\Delta H_{cal}$, on temperature. In most cases, over the limited range of temperatures which can be employed, $\Delta H_{cal}$ is found to vary linearly with temperature to within the experimental uncertainty of the DSC measurements. We report here the results of DSC measurements on bovine pancreatic ribonuclease A (RNase A) and on hen egg white lysozyme over a pH range in dilute aqueous buffer and in the presence of added sucrose, glycine, guanidinium chloride (GuCl), glycerol or sodium chloride.

## MATERIALS AND METHODS

RNase A, lysozyme, glycine, and GuCl were purchased from Sigma Chemical Co. and sucrose (analytical grade) from Mallinckrodt. The citric acid used in buffers between pH 2.8 and 7.0 was also of analytical grade. Glycine was used for buffering between pH 1.8 and 2.8. All buffers were 50 mM in concentration.

DSC was performed in an MC-2 instrument (Microcal, Inc., Northampton, MA) at a scanning rate of 60 K $h^{-1}$. The parameters $t_{1/2}$, $\Delta H_{cal}$, the true enthalpy, and $\Delta H_{vH}$, the apparent enthalpy, were evaluated by a nonlinear least-squares curve-fitting procedure (program INDEP) based on a modified two-state model in which $\Delta H_{cal}$ and $\Delta H_{vH}$ can have different values but identical temperature coefficients (Sturtevant, 1987).

## RESULTS AND DISCUSSION

A typical DSC curve is shown in Figure 1. This curve, from which the instrumental baseline determined with buffer in each cell has been subtracted, was obtained with RNase A at a concentration of 2.29 mg $mL^{-1}$ at pH 3.0 in the presence of 1.0 M sucrose. The linear pre- and posttransition baselines were evaluated by linear least squaring of the data between the indicated temperatures. The solid curve is the observed data, the open circles are the calculated points, and the dashed curve is the chemical baseline calculated by changing from the pretransition to the posttransition linear baselines in proportion to the extent of the transition. A value for $\Delta C_p$, the change in specific heat accompanying the unfolding, is obtained from the difference in height of the two linear baselines. Since these baselines in general have different slopes, $\Delta C_p$ is indicated to vary with temperature and this variation is included in the curve-fitting program. In many cases the value of $\Delta C_p$ obtained in this way is a much stronger function of temperature than it appears to be in this case, whereas plots of $\Delta H_{cal}$ vs $t_{1/2}$ similar to the one in Figure 2 indicate temperature-independent $\Delta C_p$'s. We have no explanation for this frequently occurring discrepancy [cf. Wintrode et al. (1994)].

† This research was supported in part by grants from the National Institutes of Health (GM-04725-39) and the National Science Foundation (MCB-9120192).

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⊗ Abstract published in *Advance ACS Abstracts,* February 15, 1996.

0006-2960/96/0435-3059$12.00/0 © 1996 American Chemical Society

Table 1: Thermodynamic Parameters for the Thermal Unfolding of RNase A in Buffer

| pH | concn (mg mL$^{-1}$) | $t_{1/2}$ (°C) | $\Delta H_{cal}$ (kcal mol$^{-1}$) | $\Delta H_{vH}$ (cal K$^{-1}$ mol$^{-1}$) | $\Delta H_{vH}/\Delta H_{cal}$[a] | $\Delta C_p$[b] (cal K$^{-1}$ mol$^{-1}$) | stand. deviation (% $C_{max}$) |
|---|---|---|---|---|---|---|---|
| 1.80 | 2.33 | 32.56 | 57.40 | 77.93 | 1.36 | 718 | 1.1 |
| 2.00 | 2.20 | 34.92 | 62.06 | 78.94 | 1.27 | 1328 | 1.3 |
| 2.20 | 2.15 | 38.14 | 62.06 | 87.43 | 1.41 | 416 | 0.7 |
| 2.40 | 2.20 | 41.42 | 67.54 | 87.26 | 1.29 | 534 | 0.4 |
| 2.50 | 2.03 | 45.82 | 82.47 | 90.36 | 1.10 | 704 | 1.2 |
| 2.60 | 2.23 | 44.97 | 73.57 | 90.35 | 1.23 | 415 | 0.4 |
| 2.75 | 2.11 | 48.51 | 83.79 | 95.41 | 1.14 | 38 | 0.7 |
| 2.80 | 2.14 | 48.03 | 76.31 | 93.44 | 1.22 | 940 | 0.4 |
| 3.00 | 2.26 | 50.52 | 86.86 | 100.58 | 1.16 | 963 | 0.4 |
| 3.50 | 1.97 | 56.52 | 94.80 | 104.32 | 1.10 | 1077 | 0.6 |
| 3.70 | 2.03 | 58.31 | 98.09 | 105.43 | 1.08 | 1645 | 1.1 |
| 4.00 | 2.17 | 56.75 | 92.89 | 97.29 | 1.05 | 1708 | 1.2 |
| 4.50 | 2.35 | 58.67 | 96.59 | 100.82 | 1.04 | 1581 | 1.2 |
| 5.00 | 2.30 | 60.14 | 101.38 | 104.71 | 1.03 | 912 | 1.1 |
| 5.50 | 1.87 | 65.32 | 109.05 | 123.38 | 1.13 | 1258 | 0.8 |
| 6.00 | 2.19 | 64.67 | 113.71 | 122.36 | 1.08 | 1917 | 1.1 |
| 6.00 | 2.19 | 64.67 | 113.71 | 122.01 | 1.07 | 1881 | 1.1 |
| 6.50 | 2.02 | 64.16 | 115.35 | 123.77 | 1.07 | 1334 | 0.8 |

[a] Mean 1.16, SE ± 0.03. [b] Values for $\Delta C_p$ evaluated at $t_{1/2}$ from individual scans. Mean 1080, SE ± 140.

Table 2: Summary of the Thermodynamic Parameters for the Thermal Unfolding of RNase A in Buffer and in the Presence of Sucrose, Glycine, Glycerol, GuCl, or NaCl[a]

| added solute | no. of expts | pH range | $\Delta H_{cal}$ at 0 °C (kcal mol$^{-1}$) | $\Delta C_p$ (kcal K$^{-1}$ mol$^{-1}$) | $\Delta H_{vH}/\Delta H_{cal}$ | mean stand. deviation (% $C_{max}$) |
|---|---|---|---|---|---|---|
| No added solute | 18 | 1.8−6.5 | −1.02 | 1.74 ± 0.02 | 1.16 ± 0.04 | 0.8 |
| 0.5 M sucrose | 14 | 2.2−6.5 | −66.67 | 2.70 ± 0.03 | 1.27 ± 0.08 | 1.0 |
| 1.0 M sucrose | 12 | 3.0−6.5 | −119.9 | 3.25 ± 0.02 | 1.31 ± 0.06 | 0.8 |
| 1.0 M glycine | 34 | 3.6−7.0 | −31.50 | 2.04 ± 0.02 | 1.15 ± 0.02 | 0.6 |
| 0.5 M GuCl | 23 | 3.2−6.0 | −17.87 | 1.93 ± 0.02 | 1.18 ± 0.02 | 0.7 |
| 1.0 M GuCl | 8 | 3.5−6.0 | −43.33 | 2.10 ± 0.18 | 1.62 ± 0.06 | 0.8 |
| 10% (v/v) glycerol | 15 | 2.5−6.0 | −31.54 | 1.90 ± 0.16 | 1.55 ± 0.10 | 0.5 |
| 0.5 M NaCl | 11 | 2.8−6.0 | −23.14 | 1.84 ± 0.15 | 1.33 ± 0.06 | 0.4 |

[a] Uncertainties listed are standard deviations ($\Delta C_p$ values) and standard errors of the mean ($\Delta H_{vH}/\Delta H_{cal}$).

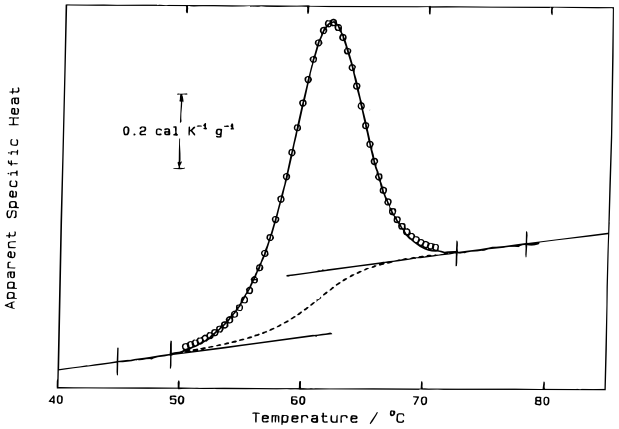


FIGURE 1: DSC trace for RNase A at a concentration of 2.29 mg mL$^{-1}$ in 50 mM phosphate buffer at pH 3.0 in the presence of 1.0 M sucrose. Scan rate 1 K min$^{-1}$. The solid curve is drawn through the observed data points and the open circles are the calculated data points. The dashed curve is the chemical baseline obtained by proceeding from the least squared pretransition baseline to the least squared posttransition baseline in proportion to the extent of the reaction.

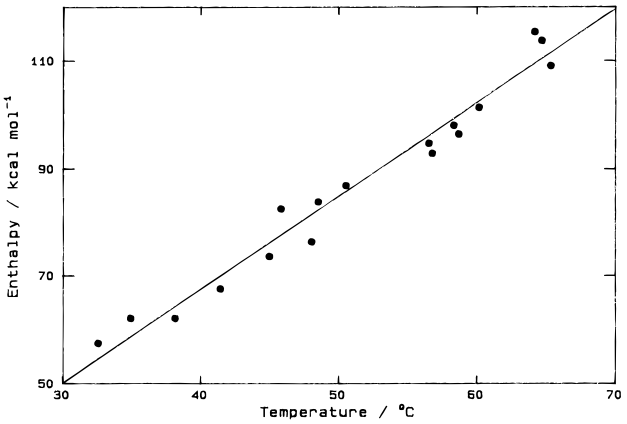


FIGURE 2: Molar enthalpy values observed for RNase A in 50 mM phosphate buffer plotted vs the temperatures of half-completion of the unfolding transitions. The solid line is the least squared line $\Delta H = -2.10 + 1.74t_{1/2}$. The points differ from this line with a standard deviation of 3.29 kcal mol$^{-1}$ (correlation coefficient = 0.970).

The results obtained in 18 runs with RNase A in buffer are summarized in Table 1. The values of $\Delta C_p$ at $t_{1/2}$ given in the seventh column were evaluated in the manner illustrated in Figure 1. As is usual with values for $\Delta C_p$ obtained in this way from individual scans, a large scatter from scan to scan occurs and the data are not of use for obtaining reliable values for $\Delta C_p$. The values of $\Delta H_{cal}$ are plotted against $t_{1/2}$ in Figure 2, illustrating the usual level of linearity for such plots.

The data obtained in DSC scans of RNase A in the absence of added solutes and in the presence of 0.5 M or 1.0 M sucrose, 1.0 M glycine, 0.5 M and 1.0 M GuCl, 10% glycerol, or 0.5 M NaCl are summarized in Table 2. The $\Delta C_p$ values in the fifth column, obtained by linear least squaring of $\Delta H_{cal}$ vs $t_{1/2}$, show standard errors of a few percent.

The rather large values for the ratio $\Delta H_{vH}/\Delta H_{cal}$ listed in column 6 indicate significant levels of aggregation of RNase A, particularly in 1.0 M sucrose. No significant variations of protein concentration were carried out, so we have no

Table 3: Summary of the Thermodynamic Parameters for the Thermal Unfolding of Lysozyme in Buffer and in the Presence of Sucrose, Glycerol, or GuCl[a]

| added solute | no. of expts | pH range | $\Delta H_{cal}$ at 0 °C (kcal mol$^{-1}$) | $\Delta C_p$ (kcal K$^{-1}$ mol$^{-1}$) | $\Delta H_{vH}/\Delta H_{cal}$[b] | mean stand. deviation (% $C_{max}$) |
|---|---|---|---|---|---|---|
| no added solute | 13 | 2.8−4.0 | 4.65 | 1.71 ± 0.27 | 0.90 ± 0.03 | 0.7 |
| 0.5 M sucrose | 12 | 2.6−4.0 | −76.87 | 2.73 ± 0.34 | 1.02 ± 0.07 | 0.5 |
| 1.0 M sucrose | 9 | 3.6−5.0 | NA | NA | 1.10 ± 0.08 | 0.9 |
| 1.0 M GuCl | 22 | 2.4−6.0 | −12.68 | 1.83 ± 0.09 | 0.99 ± 0.03 | 0.7 |
| 2.0 M GuCl | 15 | 3.2−6.0 | −10.67 | 1.69 ± 0.21 | 1.01 ± 0.03 | 0.7 |
| 10% (v/v) glycerol | 20 | 2.5−5.0 | −37.04 | 2.34 ± 0.19 | 0.93 ± 0.04 | 1.1 |

[a] Uncertainties listed are standard deviations ($\Delta C_p$ values) and standard errors of the mean ($\Delta H_{vH}/\Delta H_{cal}$). [b] Mean 0.99, SE ± 0.04.

indication as to whether any change in the extent of aggregation accompanied the unfolding of the protein. An outstanding feature of the data in Table 2 is the large increase in $\Delta C_p$ resulting from the presence of the added solutes, the value in 1.0 M sucrose, 3.25 kcal K$^{-1}$ mol$^{-1}$, being almost twice that in the absence of added solute, 1.74 kcal K$^{-1}$ mol$^{-1}$.

The data obtained for the thermal unfolding of lysozyme in buffer alone or in the presence of 0.5 M or 1.0 M sucrose, 1.0 or 2.0 M GuCl, or 10% glycerol are listed in Table 3. Actually the temperature range achieved in the presence of 1.0 M sucrose was too small to permit accurate evaluation of $\Delta C_p$. The values for $\Delta C_p$ in the fifth column of the table, obtained by linear least squaring of $\Delta H$ vs $t_{1/2}$, are of somewhat lower accuracy than those for RNase A because of the more restricted temperature ranges available. The values for the ratio $\Delta H_{vH}/\Delta H_{cal}$ in column 6 indicate that the thermal unfolding of lysozyme is a two-state process with no complication due to the aggregation of the protein. As with the data for RNase A in Table 2, we see here significant variations of $\Delta C_p$ with solution compositions.

Woolfson et al. (1993) have reported much more pronounced effects of solvent composition on the value of $\Delta C_p$ for the thermal unfolding of ubiquitin. These authors, using as we did pH changes to cause changes in the temperature of unfolding of the protein, found steady decreases in $\Delta C_p$ with increasing methanol concentrations until $\Delta C_p \approx 0$ at 30% (v/v) methanol, remaining at that value with further increases in the methanol concentration up to 50% (v/v).

Experiments reported by Santoro et al. (1992) show that varying values for $\Delta C_p$ can also result if the temperature of unfolding is changed by means other than change of pH. Thus $t_{1/2}$ for the unfolding of RNase A in 20 mM citrate buffer, pH 6, containing 55 mM NaCl increased with increasing sarcosine concentration from approximately 65.5 °C with no sarcosine present to 86.5 °C in the presence of 8.2 M sarcosine, with no detectable change in $\Delta H_{cal}$, corresponding to $\Delta C_p = 0$. The increase in the stability of the protein caused by 8.2 M sarcosine, amounting to 7.2 kcal mol$^{-1}$ free energy at 65.5 °C, is thus an entirely entropic effect. Similar effects were caused by large increases in the concentrations of other osmolytes and were also observed in the unfolding of lysozyme (Santoro et al., 1992).

Plaza del Pino and Sanchez-Ruiz (1995) have recently studied the effect of added sarcosine on the value of $\Delta C_p$ for RNase A determined at each sarcosine concentration by varying the pH. They found that $\Delta C_p$ increased with increasing sarcosine concentration according to

$$\Delta C_p \text{ (kJ K}^{-1}\text{ mol}^{-1}) = 5.30 \pm 0.0139\, m_3^{\,3}$$

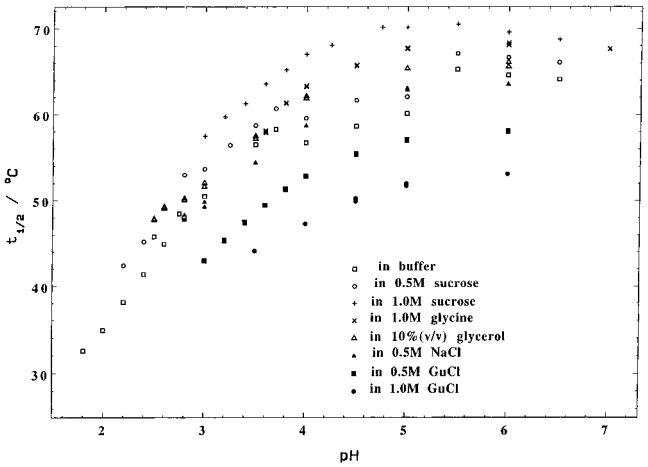


FIGURE 3: Variation with pH of the temperature of half-completion of the thermal unfolding of RNase A in the presence of various added solutes.

where $m_3$ is the molality of sarcosine. Thus the change from $m_3 = 0$ to $m_3 = 7$ was 1.27 to 2.41 kcal K$^{-1}$ mol$^{-1}$.

The value of $t_{1/2}$ for protein thermal unfolding can also be altered by the addition of salts. Hu et al. (1992) found that increasing $t_{1/2}$ for the unfolding of ribonuclease T1 (RNase T1) in various 30 mM buffers from pH 2.2 to 10.00 gave $\Delta H_{cal}$ increases corresponding to $\Delta C_p = 1.59$ kcal K$^{-1}$ mol$^{-1}$, whereas increasing $t_{1/2}$ by the addition of NaCl up to 1.6 M at pH 7 led to $\Delta C_p = 1.10$ kcal K$^{-1}$ mol$^{-1}$, and increasing $t_{1/2}$ by the addition of $MgCl_2$ up to 0.8 M gave $\Delta C_p = 1.14$ kcal K$^{-1}$ mol$^{-1}$.

Observations such as those outlined here indicate that the value of $\Delta C_p$ is a function of the circumstances under which the variation of $\Delta H_{cal}$ with respect to $t_{1/2}$ is observed. It does not seem that any method employed for changing $t_{1/2}$ is fundamentally more appropriate than any other method, or that any particular value obtained for $\Delta C_p$ is a more fundamental quantity than one obtained in some other solvent composition or by some other procedure for changing $t_{1/2}$.

The variation of $t_{1/2}$ with pH is shown in Figure 3 for RNase A and in Figure 4 for lysozyme, in each case in all the solvent systems used in the present work. The denaturational change, $\Delta n$, in the number of protons bound to the protein is given by eq 1, which is a form of the van't Hoff equation:

$$\Delta n = \frac{\Delta H}{2.303\, RT_{1/2}^{\,2}} \frac{\mathrm{d}T_{1/2}}{\mathrm{d\ pH}} \qquad (1)$$

where $T_{1/2} = t_{1/2} + 273.15$ and $\Delta H$ is expressed in calories per mole.

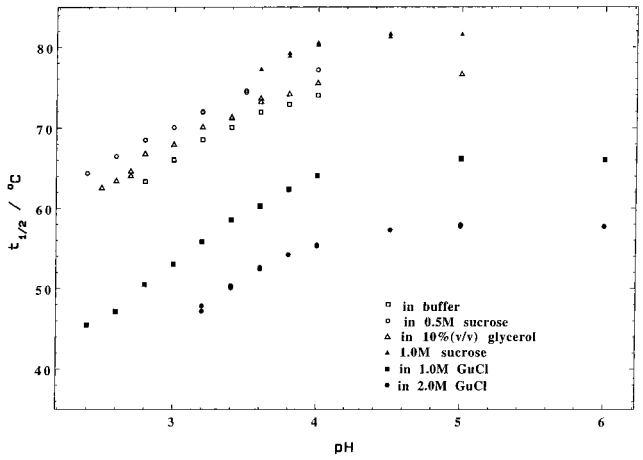


FIGURE 4: Variation with pH of the temperature of half-completion of the thermal unfolding of lysozyme in the presence of various added solutes.

Table 4: Variation of the Free Energy of Thermal Unfolding of RNase A with Temperature at pH 4, 5, and 6 in the Presence of Various Added Solutes

| | $\Delta G°$ (kcal mol$^{-1}$) | | | | |
|---|---|---|---|---|---|
| added solute | 10 °C | 25 °C | 40 °C | 55 °C | $t_{1/2}$ (°C) |
| | | pH = 4.0 | | | |
| none | 7.79 | 6.66 | 4.21 | 0.51 | 56.75 |
| 0.5 M sucrose | 3.53 | 4.77 | 3.96 | 1.22 | 59.6 |
| 1.0 M sucrose | −0.06 | 3.30 | 4.20 | 2.77 | 67.1 |
| 1.0 M glycine | 6.36 | 6.49 | 5.08 | 2.20 | 63.3 |
| 0.5 M GuCl | 10.07 | 7.88 | 4.23 | −0.80 | 52.85 |
| 1.0 M GuCl | 1.77 | 2.22 | 1.09 | −1.54 | 47.3 |
| 10% (v/v) glycerol | 5.31 | 5.51 | 4.28 | 1.69 | 62.1 |
| 0.5 M NaCl | 5.53 | 5.36 | 3.80 | 0.91 | 58.7 |
| | | pH = 5.0 | | | |
| none | 8.67 | 7.58 | 5.18 | 1.53 | 60.1 |
| 0.5 M sucrose | 4.15 | 5.42 | 4.65 | 1.93 | 62.1 |
| 1.0 M sucrose | 0.72 | 4.12 | 5.07 | 3.68 | 70.2 |
| 1.0 M glycine | 7.47 | 7.66 | 6.30 | 3.48 | 67.7 |
| 0.5 M GuCl | 11.44 | 9.33 | 5.75 | 0.79 | 57.1 |
| 1.0 M GuCl | 2.52 | 3.02 | 1.93 | −0.67 | 51.85 |
| 10% (v/v) glycerol | 6.05 | 6.30 | 5.10 | 2.55 | 65.4 |
| 0.5 M NaCl | 6.50 | 6.38 | 4.87 | 2.03 | 63.0 |
| | | pH = 6.0 | | | |
| none | 9.90 | 8.87 | 6.54 | 2.95 | 64.7 |
| 0.5 M sucrose | 5.37 | 6.70 | 6.00 | 3.35 | 66.7 |
| 1.0 M sucrose | 0.57 | 3.97 | 4.90 | 3.51 | 69.6 |
| 1.0 M glycine | 7.60 | 7.79 | 6.45 | 3.63 | 68.2 |
| 0.5 M GuCl | 11.79 | 9.69 | 6.13 | 1.19 | 58.1 |
| 1.0 M GuCl | 2.76 | 3.27 | 2.19 | −0.40 | 53.2 |
| 10% (v/v) glycerol | 6.14 | 6.39 | 5.20 | 2.65 | 65.8 |
| 0.5 M NaCl | 6.64 | 6.52 | 5.02 | 2.19 | 63.6 |

It is evident that in each system there is little or no deviation from $\Delta n = 0$ at pH 5−7 but values significantly greater than zero at low pH, as should be expected. Values for $\Delta n$ at pH 3 range for RNase A from 2.9 in buffer to 1.3 in the presence of 1 M sucrose, and for lysozyme from 2.7 in buffer to 1.9 in the presence of 0.5 M sucrose.

Tables 4 and 5 list the standard free energies of unfolding of RNase A and lysozyme, respectively, in the absence and the presence of the various added solutes studied in this work. These quantities were calculated using the Gibbs−Helmholtz equation:

$$\P\l\Delta G°_T = \Delta H_{1/2}\,(1 - T/T_{1/2}) - \Delta C_p[T_{1/2} - T + T \ln (T/T_{1/2})] \quad (2)$$

Table 5: Variation of the Free Energy of Thermal Unfolding of Lysozyme with Temperature at pH 3, 4, 5, and 6 in the Presence of Various Added Solutes

| | $\Delta G°$ (kcal mol$^{-1}$) | | | | |
|---|---|---|---|---|---|
| added solute | 25 °C | 40 °C | 55 °C | 70 °C | $t_{1/2}$ (°C) |
| | | pH = 3.0 | | | |
| none | 9.80 | 7.27 | 3.51 | −1.42 | 66.0 |
| 0.5 M sucrose | 6.56 | 6.32 | 4.10 | 0.02 | 70.1 |
| 1.0 M GuCl | 4.97 | 2.88 | −0.51 | −5.16 | 53.05 |
| 10% (v/v) glycerol | 8.73 | 7.22 | 4.03 | −0.76 | 67.9 |
| | | pH = 4.0 | | | |
| none | 12.30 | 9.90 | 6.27 | 1.47 | 74.0 |
| 0.5 M sucrose | 8.74 | 8.60 | 6.50 | 2.53 | 77.1 |
| 1.0 M sucrose | 6.64 | 7.28 | 6.06 | 3.05 | 80.3 |
| 1.0 M GuCl | 7.78 | 5.83 | 2.58 | −1.93 | 64.1 |
| 2.0 M GuCl | 5.23 | 3.27 | 0.09 | −4.25 | 55.35 |
| 10% (v/v) glycerol | 11.19 | 9.81 | 6.74 | 2.08 | 75.44 |
| | | pH = 5.0 | | | |
| 1.0 M sucrose | 7.01 | 7.67 | 6.47 | 3.48 | 81.6 |
| 1.0 M GuCl | 8.36 | 6.45 | 3.22 | −1.26 | 66.2 |
| 2.0 M GuCl | 5.81 | 3.88 | 0.73 | −3.58 | 57.9 |
| 10% (v/v) glycerol | 11.58 | 10.22 | 7.17 | 2.52 | 76.6 |
| | | pH = 6.0 | | | |
| 1.0 M GuCl | 8.34 | 6.42 | 3.19 | −1.28 | 66.1 |
| 2.0 M GuCl | 5.79 | 3.85 | 0.70 | −3.61 | 57.75 |

where $\Delta H_{1/2}$ is the value of $\Delta H_{cal}$ at $T_{1/2}$ and $\Delta G° = 0$ for a process of the form A ⇄ B, and they illustrate the importance of $\Delta C_p$. Of course, the accuracy of the values for $\Delta G°$ at temperatures far removed from $t_{1/2}$ suffer from uncertainties in $\Delta C_p$. This is particularly true in the case of lysozyme in the presence of 1 M sucrose.

A number of values of $\Delta C_p$ for RNase A unfolding under various conditions have been reported by other authors. These cover the range 1.0 kcal K$^{-1}$ mol$^{-1}$ (Jacobsen & Turner, 1980) to 2.2 kcal K$^{-1}$ mol$^{-1}$ (Salahuddin & Tanford, 1970). For lysozyme, the corresponding range is 1.34 kcal K$^{-1}$ mol$^{-1}$ (Fujita et al., 1982) in buffer to 3.0 kcal K$^{-1}$ mol$^{-1}$ in 8 M urea (Makhatadze & Privalov, 1992).

## ACKNOWLEDGMENT

We are indebted to Drs. S.-J. Bae, J. Ladbury, A. Tamura, and J. Thomson for helpful discussions.

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BI952198J