Urea and Guanidine Hydrochloride Denaturation of Ribonuclease, Lysozyme, α-Chymotrypsin, and β-Lactoglobulin*

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SUMMARY
The unfolding of ribonuclease, lysozyme, α-chymotrypsin, and goat β-lactoglobulin by urea and guanidine hydrochloride (GmHCl) has been followed with the use of optical rotation measurements. Urea denaturation leads to a more negative rotation for each protein than does GmHCl denaturation, but the concentration dependence is such that the rotations are almost identical in the absence of denaturant. This indicates that both urea and GmHCl denaturation lead to a similar, randomly coiled conformation. By assuming a two-state mechanism, an apparent free energy of unfolding, \( \Delta G_{\text{app}} \), has been calculated as a function of denaturant concentration. \( \Delta G_{\text{app}} \) varies linearly with denaturant concentration. The dependence of \( \Delta G_{\text{app}} \) on concentration ranges from 1.1 to 2.1 Cal per mole per molar concentration for urea and from 1.9 to 4.1 Cal per mole per molar concentration for GmHCl for the four proteins. These values depend on the size and composition of the part of the polypeptide chain which is freshly exposed to denaturant by unfolding. By using model compound data and a procedure developed by Tanford (Adv. Protein Chem. 1970, 24, 1-95), an estimate of the amount of buried polypeptide chain needed to account for the experimental results has been obtained. The estimates based on urea and on GmHCl are in excellent agreement. This offers further support for the idea that the extent of unfolding is the same for the two denaturants and also suggests that the mechanism of unfolding is the same in the presence of the two denaturants. The relative effectiveness of the two denaturants depends on the protein. GmHCl is 2.8 times more effective than urea in unfolding ribonuclease but only 1.7 times more effective for lysozyme. This results because the buried polypeptide chain is more polar for ribonuclease than for lysozyme and the solubilizing ability of GmHCl relative to urea is greater for polar groups than for nonpolar groups. Estimates of \( \Delta G_{\text{app}} \) in the absence of denaturant, \( \Delta G_{\text{H2O}} \), were obtained from the analysis with the use of model compound data and also from a direct linear extrapolation of \( \Delta G_{\text{app}} \) to zero concentration of denaturant. For urea, the two approaches lead to values of \( \Delta G_{\text{H2O}} \) in reasonably good agreement. For GmHCl, only the values of \( \Delta G_{\text{app}} \) from the direct extrapolation are in good agreement with the results from urea. The \( \Delta G_{\text{H2O}} \) values obtained are 9.7 ± 1.7 Cal per mole for ribonuclease at pH 6.6, 6.1 ± 0.4 for lysozyme at pH 2.9, 8.3 ± 0.4 for α-chymotrypsin at pH 4.3, and 11.7 ± 0.8 for β-lactoglobulin at pH 3.2. These results suggest that several recent estimates of \( \Delta G_{\text{app}} \) are too high by 3 to 10 Cal per mole.

The native conformation of most globular proteins is only marginally more stable (5 to 15 Cal per mole) than a randomly coiled conformation under physiological conditions (1). It would be useful to have reliable estimates of this stability for a representative sample of globular proteins. However, these estimates are difficult to obtain, and at present only a few such estimates are available (1-8). These estimates are usually based on a study of the denaturation with a single denaturant, and the uncertainty is high. A major problem is extrapolating from the conditions used for unfolding the protein to physiological conditions. To gain a better understanding of this area, we have studied the urea and guanidine hydrochloride denaturation, under identical conditions, of four proteins: ribonuclease, lysozyme, α-chymotrypsin, and goat β-lactoglobulin. These proteins present a range of molecular weights and polarities and, for each, the denaturation has been investigated previously in some detail. The ability of urea to denature proteins was first reported about 1900 by Spiro (9) and Ramsden (10). (Ramsden’s interest was not confined to “proteids,” for he also reported: “A dead frog placed in saturated urea solution becomes translucent and falls to pieces in a few hours.”) The even greater effectiveness of guanidine hydrochloride (GmHCl) as a protein denaturant was first noted by Greenstein in 1938 (11). At present, urea and GmHCl are the most frequently used protein denaturants. The main advantage of these denaturants is that the extent of unfolding is generally greater than can be achieved by other means of denaturation. Despite their widespread use, few comparative studies on urea and GmHCl as protein denaturants have been reported. Our results provide such a comparison.

EXPERIMENTAL PROCEDURES
Goat β-lactoglobulin was prepared from the milk of two Saanen goats by the procedure of Kalan and Baech (12). Ribonuclease, lysozyme, α-chymotrypsin, and goat β-lactoglobulin were prepared as described previously (13). The denaturation was followed by determining the optical rotation of the protein solutions in the presence of urea or GmHCl. The \( \Delta G_{\text{app}} \) values were then calculated using the procedure of Tanford (14). The results obtained with urea and GmHCl are in good agreement, and the \( \Delta G_{\text{H2O}} \) values obtained are 9.7 ± 1.7 Cal per mole for ribonuclease, 6.1 ± 0.4 Cal per mole for lysozyme, 8.3 ± 0.4 Cal per mole for α-chymotrypsin, and 11.7 ± 0.8 Cal per mole for β-lactoglobulin. These results suggest that several recent estimates of \( \Delta G_{\text{app}} \) are too high by 3 to 10 Cal per mole.

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1 The abbreviation used is: GmHCl, guanidine hydrochloride.
type IIIA (Lot 99B-2080), was obtained from Sigma Chemical Co. The protein was deionized with a Dintzis column (13). Twice crystallized lysozyme (Lot LYSF OCC) and thrice crystallized cu-chymotrypsin (Lot CD1 2LX) were obtained from Worthington Biochemical Corp.

The concentration of goat β-lactoglobulin stock solutions was determined from optical rotation measurements by using the value \( [\alpha'] = -95.1 \) at 365.4 nm at pH 3. Other protein concentrations were determined spectrophotometrically by using the following extinction coefficients: ribonuclease, \( E_{365\text{nm}} = 7.2 \) (14); lysozyme, \( E_{365\text{nm}} = 26.4 \) (8); and chymotrypsin, \( E_{365\text{nm}} = 20.7 \) (15).

Ultrapure urea was purchased from Mann Research Laboratories. Stock solutions were prepared by weight, and their molarities were determined by using densities calculated from equations given by Kawahara and Tanford (16). All urea stock solutions contained 0.15 M HCl-KCl. The GmHCl was purchased from Heico, Inc. The concentration of stock solutions was determined from refractive index measurements (17).

Each of the optical rotations shown in Figs. 1 to 4 is an equilibrium measurement on a freshly prepared solution in a Cary 60 spectropolarimeter. The final protein concentrations ranged from 0.001 to 0.01 g per ml. All rotations were measured at 365.4 nm and are reported as \([\alpha']\), the reduced specific rotation (19). Values of \(3/[(n^2+2)]\) for GmHCl and urea solutions were obtained from Pace (17). The temperature was 25.0 ± 0.1°C for all measurements. The pH of solutions in the transition region was determined after the rotation had been measured.

RESULTS

The urea and GmHCl denaturation curves for ribonuclease, lysozyme, α-chymotrypsin, and goat β-lactoglobulin are shown in Figs. 1 to 4. In all cases denaturation was followed by measuring the optical rotation at 365.4 nm, so the curves are directly comparable. Urea denaturation was carried out in the presence of 0.15 M KCl-HCl to compensate partially for the differences in ionic strength which result because GmHCl is a salt. An ionic strength of 0.15 M will largely eliminate the electrostatic interactions between charged groups on the surface of globular proteins (20). Also, for each protein the denaturation curves were determined at approximately the same pH. Thus, the observed differences in the denaturation curves reflect the relative effectiveness of these two widely used protein denaturants. It is clear that GmHCl is able to unfold proteins at substantially lower
concentrations than urea but that the magnitude of the difference depends on the protein.

Reversibility—The denaturation of ribonuclease by urea (21) and GmHCl (7), of lysozyme by urea (22) and GmHCl (23), of \( \alpha \)-chymotrypsin by urea (24) and GmHCl (25), and of goat \( \beta \)-lactoglobulin by urea (19) has been shown to be completely reversible under the conditions used in this paper. We find that the GmHCl denaturation of \( \beta \)-lactoglobulin is also completely reversible.

Denatured States—Studies by Tanford’s group (26-28) have shown that proteins denatured by GmHCl closely approach a randomly coiled conformation. Less information is available for urea-denatured proteins, and it is not certain whether the product of urea denaturation is as completely unfolded as in the case of GmHCl (29). For example, for ribonuclease and \( \beta \)-lactoglobulin the intrinsic viscosities of the proteins in 6 M GmHCl are 1.8 and 2.9 ml per g, respectively, higher than in 8 M urea (30). It can be seen in Figs. 1 to 4 that the \( [\alpha'] \) values for urea-denatured proteins are more negative than for the GmHCl-denatured proteins. However, in all cases, it appears that the \( [\alpha'] \) values will become almost identical when extrapolated to zero concentration of denaturant. This is good evidence that, for these four proteins, urea and GmHCl denaturation leads to a similar, randomly coiled conformation. The differences in \( [\alpha'] \) observed at higher denaturant concentrations are probably the result of specific solvent effects on the optical rotation. The differences in the intrinsic viscosities noted above may also result from these solvent effects.

The \( [\alpha'] \) values of the denatured proteins vary from \(-241^\circ\) for lysozyme to \(-329^\circ\) for \( \beta \)-lactoglobulin. It has been shown that these differences can be accounted for solely on the basis of differences in amino acid sequence; they do not indicate a difference in conformation (30). The native rotations vary from \(-95^\circ\) for \( \alpha \)-chymotrypsin to \(-235^\circ\) for ribonuclease. These differences are, of course, a reflection of the unique conformations which the native proteins possess. Because of these differences there is only a 1.3-fold decrease in the rotation accompanying the unfolding of ribonuclease but a 3.3-fold decrease for \( \beta \)-lactoglobulin.

Free Energy of Denaturation—For ribonuclease (7, 31, 32) and lysozyme (33-35), a number of different studies have shown that urea and GmHCl denaturation approaches a two-state mechanism quite closely. For \( \alpha \)-chymotrypsin, kinetic studies of the urea and GmHCl denaturation give results that are consistent with a two-state mechanism (24, 36, 37). Less is known about the mechanism of denaturation for goat \( \beta \)-lactoglobulin, but we will assume that a two-state mechanism is applicable for this protein also (38).

For a two-state mechanism, the equilibrium constant, \( K \), and the free energy of denaturation, \( G_{\text{app}} \), can be determined from the experimental data by using

\[
K = e^{-\Delta G_{\text{app}}/RT} = \frac{[\alpha']_N - [\alpha']_D}{[\alpha']_N - [\alpha']_D}
\]

where \([\alpha']\) is the observed rotation and \([\alpha']_N\) and \([\alpha']_D\) represent the rotations which the native and denatured states would have under the same conditions. The values of \([\alpha']_N\) and \([\alpha']_D\) were taken from the linear lines through the points at high and low denaturant concentrations shown in Figs. 1 to 4. In some cases there was uncertainty in where to draw these lines, but alternate choices have only a small effect on our results.

In Fig. 5 \( \Delta G_{\text{app}} \) for the unfolding of lysozyme is seen to vary linearly with denaturant concentration. Aune and Tanford (8) (see page 61 in Ref. 1) have shown that this linear variation holds over an even wider GmHCl concentration range. Similar results were obtained for all of the proteins. A least squares analysis was used to fit data of this kind to the equation

\[
\Delta G_{\text{app}} = \Delta G_{\text{app}}^{\text{den}} - m(\text{denaturant})
\]

Values of \( \Delta G_{\text{app}}^{\text{den}} \) and \( m \) are listed in Table I along with the denaturant concentration at the midpoint of the denaturation curves, \( (\text{denaturant})_{1/2} \). The ratio of the midpoint in urea to that in GmHCl, \((\text{urea})_{1/2} / (\text{GmHCl})_{1/2} \), is 2.31 for ribonuclease, 1.70 for lysozyme, 2.13 for \( \alpha \)-chymotrypsin, and 1.55 for \( \beta \)-lactoglobulin. Note that this corresponds to a difference between the midpoint molarities of only 1.78 for \( \beta \)-lactoglobulin but 3.95 for ribonuclease. The midpoint of a denaturation curve depends on both the stability of the protein in absence of denaturant and on the dependence of the free energy of unfolding on denaturant concentration. Thus, the ability of a denaturant to unfold a protein is more directly reflected in the \( m \) value. The ratio of the \( m \) values for the two denaturants, \( m(\text{GmHCl})/m(\text{urea}) \), is 2.82 for ribonuclease, 1.68 for lysozyme, 1.18 for \( \alpha \)-chymotrypsin, and 1.84 for \( \beta \)-lactoglobulin.

### Table I

<table>
<thead>
<tr>
<th>Protein</th>
<th>pH</th>
<th>(GmHCl)_{1/2}</th>
<th>(Urea)_{1/2}</th>
<th>( \Delta G_{\text{app}}^{\text{den}} )</th>
<th>( m )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>M</td>
<td>Cal/ mol</td>
<td>Cal/ mol</td>
<td>M</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>6.6</td>
<td>3.01</td>
<td>9.3</td>
<td>3.10</td>
<td>6.96</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>2.9</td>
<td>3.07</td>
<td>5.8</td>
<td>1.88</td>
<td>5.21</td>
</tr>
<tr>
<td>( \alpha )-Chymotrypsin</td>
<td>4.3</td>
<td>1.90</td>
<td>7.8</td>
<td>4.10</td>
<td>4.04</td>
</tr>
<tr>
<td>Goat ( \beta )-lactoglobulin</td>
<td>3.2</td>
<td>3.23</td>
<td>12.5</td>
<td>3.87</td>
<td>5.01</td>
</tr>
</tbody>
</table>

\( a \) The midpoint \((\text{GmHCl})_{1/2} = 0\) of the denaturation curve.

\( b \) From Equation 2.
**DISCUSSION**

The values of \(m\) in Table I measure the dependence of \(\Delta G_{\text{app}}\) on denaturant concentration. They depend, then, on the steepness of the denaturation curve and are clearly a more useful and meaningful parameter than the slope of a plot of \(\log(K_{\text{app}})\) versus \(\log(\text{denaturant})\), which has often been used in the past as a measure of the steepness of denaturation curves. Tanford (1) has developed a procedure for calculating \(m\) which is based on model compound data. Since the required model compound data are available for both GmHCl and urea, our data should provide a good test of the applicability of this procedure. In addition, we will gain a useful insight into what determines the value of \(m\) for a given protein and denaturant.

From solubility studies on amino acids and related derivatives, the free energies of transfer from water to various concentrations of denaturant have been calculated for the component parts of a protein (39, 40). With this information, Tanford (1) has shown that a value of \(m\) can be calculated if the number and type of residues which are freshly exposed to denaturant by the unfolding process are known. The necessary relationship is

\[
\Delta G_{\text{app}} = \Delta G_{\text{app}}^{\text{id}} - \sum \alpha \delta g_{\text{tr}},
\]

where \(\Delta G_{\text{app}}^{\text{id}}\) is the free energy of unfolding in water, \(\alpha\) is the average change in the degree of exposure to solvent during unfolding of groups of type \(i\), and \(\delta g_{\text{tr}},\) is the free energy of transfer for groups of type \(i\) from one solvent composition to another. Values of \(\delta g_{\text{tr}},\) for the side chains are taken from Nosaki and Tanford (39, 40). All of the side chains except those of aspartic acid, glutamic acid, lysine, arginine, and serine were included in the calculations. Values of \(\delta g_{\text{tr}},\) for a peptide group are given in Table II. These values are based on solubility studies on N-acetyltetraglycine ethyl ester and ethyl acetate, reported by Robinson and Jencks (41). They are reported here since all of the \(\delta g_{\text{tr}}\) values for urea have not been published previously.

By using Equation 3 we have determined a single average value of \(\alpha\) for all of the groups, denoted \(\bar{\alpha}\), which leads to a calculated value of \(m\) identical with that determined experimentally. The values of \(\bar{\alpha}\) so determined are given in Table III, along with the values of \(\Delta G_{\text{app}}\) calculated from these \(\bar{\alpha}\) values. The values of \(\bar{\alpha}\) from the two denaturants are in excellent agreement, considering the approximate approach used in the calculations and the uncertainty in the values of \(\delta g_{\text{tr}}\), for a peptide group. This agreement offers further proof that the extent of unfolding produced by the two denaturants is approximately the same. In addition, the agreement suggests that the mechanism of denaturation is similar for the two denaturants.

The agreement between the \(\bar{\alpha}\) values for the two denaturants also points out that the relative effectiveness of the two denaturants depends on the makeup of the portion of the chain which is exposed to denaturant on unfolding. The \(\delta g_{\text{tr}}\) values for peptide groups and polar side chains are from 2.4 to 2.9 times larger for GmHCl than for urea, whereas the values for nonpolar side chains are only from 1.6 to 2.3 times greater (40). Thus, the reason \(m\) (GmHCl)/\(m\) (urea) = 2.8 for ribonuclease and only 1.7 for lysozyme is that the unit which unfolds is considerably more nonpolar for lysozyme than for ribonuclease.

In general, \(\bar{\alpha}\) and \(m\) should increase with increasing molecular weight because the fraction of the residues buried is expected to increase. The molecular weights of the proteins are given in Table III, and it is clear that the \(\bar{\alpha}\) values do not correlate with molecular weight. The largest protein, \(\alpha\)-chymotrypsin, has the smallest value of \(\bar{\alpha}\) and the two proteins with similar molecular weights, ribonuclease and lysozyme, have significantly different \(\bar{\alpha}\) values. The most likely explanations for these differences in the \(\bar{\alpha}\) values are: (a) the relative accessibilities of the native states of the proteins differ; (b) the relative accessibilities of the denatured states of the proteins differ; (c) our method of analysis in which the peptide groups and the side chains are lumped together is at fault.

Lee and Richards (42) have analyzed the accessibility of atoms in native lysozyme and ribonuclease S. They find that native ribonuclease S is somewhat more accessible to solvent than lysozyme. Moreover, when their accessibilities are used to assign an \(\alpha\) value for each type of side chain, the difference between the \(\bar{\alpha}\) values for lysozyme and ribonuclease S becomes even greater. Consequently, the first and third explanations given above cannot account for the difference between the \(\bar{\alpha}\) values for these two proteins.

A large part of the difference in the \(\bar{\alpha}\) values for the proteins probably results from differences in the accessibility of the denatured states. The intrinsic viscosities of the four proteins in 6 M GmHCl, with and without their disulfides broken, are given in Table III (30). Data for \(\alpha\)-chymotrypsin are not available, but Biltonen (43) has shown that the properties of the denatured states of \(\alpha\)-chymotrypsin and chymotrypsinogen are very similar. On breaking the disulfide bonds the percentages of change in the

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**Table II**

<table>
<thead>
<tr>
<th>Peptide group</th>
<th>(\delta g_{\text{tr}}^0) cal/ mole</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>90</td>
</tr>
<tr>
<td>2</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td>118</td>
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<tr>
<td>6</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>90</td>
</tr>
<tr>
<td>GmHCl</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>83</td>
</tr>
<tr>
<td>2</td>
<td>134</td>
</tr>
<tr>
<td>4</td>
<td>207</td>
</tr>
<tr>
<td>6</td>
<td>245</td>
</tr>
</tbody>
</table>

\(\delta g_{\text{tr}}\) was calculated from the solubility data of Robinson and Jencks (41), on N-acetyltetraglycine ethyl ester and ethyl acetate, as described by Nosaki and Tanford (40).

---

**Table III**

<table>
<thead>
<tr>
<th>Protein</th>
<th>(\bar{\alpha})</th>
<th>(\Delta G_{\text{app}}^{\text{id}}) cal/mole</th>
<th>(\bar{\alpha})</th>
<th>(\Delta G_{\text{app}}^{\text{id}}) cal/mole</th>
<th>Gal/mol</th>
<th>Gal/mol</th>
<th>ml/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ribonuclease.....</td>
<td>0.35</td>
<td>14.8</td>
<td>0.34</td>
<td>12.1</td>
<td>13,700</td>
<td>9.4</td>
<td>16.3</td>
</tr>
<tr>
<td>Lysozyme.........</td>
<td>0.20</td>
<td>9.1</td>
<td>0.21</td>
<td>6.6</td>
<td>14,300</td>
<td>6.5</td>
<td>17.1</td>
</tr>
<tr>
<td>(\alpha)-Chymotrypsin</td>
<td>0.17</td>
<td>10.4</td>
<td>0.21</td>
<td>8.8</td>
<td>25,000</td>
<td>11.0</td>
<td>(28.8)</td>
</tr>
<tr>
<td>(\beta)-Lactoglobulin..</td>
<td>0.33</td>
<td>18.4</td>
<td>0.34</td>
<td>12.1</td>
<td>18,400</td>
<td>10.1</td>
<td>22.8</td>
</tr>
</tbody>
</table>

* From Equation 3.

* Intrinsic viscosity in 6 M GmHCl (30).

* Data for chymotrypsinogen.
intrinsic viscosities are 62% for lysozyme, 59% for chymotrypsinogen, 42% for ribonuclease, and 16% for β-lactoglobulin. Since ribonuclease contains relatively more disulfide bonds than do the other proteins, it is clear that these differences result in part from the location of the disulfide bonds. The more compact denatured state possessed by lysozyme and α-chymotrypsin probably reduces the accessibility to denaturant and accounts at least in part for the lower values of $\bar{\alpha}$ observed for these two proteins.

We have assumed in the discussion above that the two-state mechanism used in analyzing the data is valid. If significant concentrations of intermediate states are present, the observed values of $m$ will almost always be less than the $m$ value expected for a two-state transition (30). This offers another possible explanation for differences in the $\bar{\alpha}$ values.

It is difficult to obtain a reliable estimate of the stability of a globular protein under physiological conditions. No matter what techniques are used for destabilizing the protein so that the equilibrium between native and unfolded states can be studied, there is a sizable extrapolation to physiological conditions. For urea and GmHCl denaturation the problem is evident from Fig. 5; measurements of $\Delta G_{\text{app}}^0$ are possible only at higher denaturant concentrations. One estimate of the stability in water, $\Delta G_{\text{app}}^0$, is given in Table 1. The assumption here is that the linear dependence of $\Delta G_{\text{app}}^0$ on denaturant concentration continues to zero concentration of denaturant. A higher estimate is obtained by using an extrapolation based on the model compound data and is given in Table III. A still higher estimate is obtained if the extrapolation is based on models which assume that denaturation results because the denatured state possesses a greater number of denaturant binding sites than the native state (1, 3, 7, 8).

At present it is not clear which extrapolation procedure leads to a better estimate of $\Delta G_{\text{app}}^0$. For the urea denaturation of ribonuclease and chymotrypsinogen, measurements of $\Delta G_{\text{app}}^0$ have been extended to zero concentration of urea by destabilizing the protein by lowering the pH. For ribonuclease, $\Delta G_{\text{app}}^0$ varies linearly to zero urea concentration (44). For chymotrypsinogen the variation is linear to 1.5 $M$, but the $m$ value then decreases (45); i.e. the $m$ value varies in a direction opposite to that expected on the basis of the model compound data. These results are not conclusive, however, because it has been shown that the product of acid denaturation is less completely unfolded than the product of urea or GmHCl denaturation for both ribonuclease and chymotrypsinogen (46). Consequently, at low concentrations of urea the denatured state probably possesses some folded structure, and a decrease in the value of $m$ is expected. These studies offer some support to estimates of $\Delta G_{\text{app}}^0$ based on the direct extrapolation procedure. However, Puett (3) obtained reasonable agreement between values of $\Delta G_{\text{app}}^0$ from acid, urea, and GmHCl denaturation, using Tanford’s procedure (1) or the denaturant binding model to obtain $\Delta G_{\text{app}}^0$ from the urea and GmHCl data.

Our studies support the direct extrapolation procedure. For urea, the values of $\Delta G_{\text{app}}^0$ in Tables I and III are in reasonable agreement except in the case of ribonuclease. For GmHCl, the values of $\Delta G_{\text{app}}^0$ obtained by using the model compound data (Table III) are from 2.0 to 5.9 Cal per mole higher than those based on direct extrapolation (Table I). The agreement between values of $\Delta G_{\text{app}}^0$ from the two denaturants is quite good when direct extrapolation is used but much less satisfactory when the model compound data are used. The failure of Tanford’s procedure (1) to give a consistent result in the case of GmHCl may be related to an ionic strength effect on the model compound.

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