Guanidine Hydrochloride Unfolding of Peptide Helices: Separation of Denaturant and Salt Effects†

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ABSTRACT: To provide a model for understanding the unfolding of proteins by the chemical denaturant guanidine hydrochloride, we have measured helix unfolding for homologous series of peptides with the repeating sequence Ala-Glu-Ala-Ala-Lys-Ala and chain lengths from 7 to 50 residues. The free energy for helix unfolding varies as a function of guanidinium chloride (GdmCl) for all the peptides. The slope of the linear plot of the free energy of helix formation as a function of the molar concentration of GdmCl, termed the *m*-value, was found to be strongly dependent on the total ionic strength of the solution. A comparison of the *m*-value for urea denaturation of the same series of peptides [Scholtz, J. M., Barrick, D., York, E. J., Stewart, J. M., & Baldwin, R. L. (1995) *Proc. Natl. Acad. Sci. U.S.A. 92*, 185−189] reveals that, under many conditions, GdmCl is roughly twice as effective as a denaturant than urea on a molar basis, in agreement with many studies on proteins. However, when the ionic strength of aqueous GdmCl is controlled with additional NaCl, it is possible to separate the observed *m*-value for GdmCl solutions into two components: one that is identical to that found for urea and a second which depends only on the molar concentration of the chloride anion. Therefore, for these peptides, an equimolar mixture of urea and NaCl is nearly as effective as GdmCl in unfolding the helical conformation.

Guanidinium chloride (GdmCl)[1] and urea are two agents commonly employed as protein denaturants. Analysis of solvent denaturation curves, using urea or GdmCl as denaturants, can provide a measure of the conformational stability of a protein (Pace, 1975, 1986). Despite the widespread use of solvent denaturation curves, a molecular description of the interaction of urea or GdmCl with proteins is lacking, due in part to the complex structures of folded proteins. Simple peptides that are able to adopt specific secondary structure, like the α-helix, can serve as good model systems for understanding the physical basis for the effects of denaturants on protein stability.

The observation that short, alanine-based peptides form monomeric helices in aqueous solution (Marqusee & Baldwin, 1987) has permitted the determination of the energetics of many aspects of helix formation including helix propensity measurements for the twenty amino acids that occur in proteins, determination of specific electrostatic side-chain interactions, including charge−dipole effects and helix capping parameters, the hydrophobic interactions between side chains [for reviews see Scholtz and Baldwin (1992, 1995), and Chakrabartty and Baldwin (1993, 1995)]. A recent report has used simple helical peptides as a model system to understand how urea acts as a protein denaturant (Scholtz et al., 1995). Here we investigate the effects of the other popular protein denaturant, GdmCl, on helix formation in the same set of peptides and arrive at some very interesting conclusions. At least for the peptide studied here, it appears that urea and guanidine are equally effective as helix-unfolding agents, when the ionic nature of aqueous GdmCl solutions is taken into account. The ramifications of these results for protein folding and stability studies are discussed.

MATERIALS AND METHODS

Peptides Studied. The peptides, ranging in length from 14 to 50 residues, have the general sequence AcY(AEAAKA)$_m$-F(NH$_2$), where the N- and C-termini are blocked with acetyl and carboxamide groups, respectively. The details of the design, synthesis, and purification of the peptides are described in an earlier report (Scholtz et al., 1991a). In addition, a single 7-residue peptide, AcYAEAAKA(NH$_2$), was prepared using standard FMOC chemistry and solid-phase peptide synthesis. The details of the peptide synthesis and purification methods are identical to those described earlier (Scholtz et al., 1993).

Measurement of Helix Formation. Helix content was measured by circular dichroism (CD) spectroscopy with an Aviv 62DS spectropolarimeter equipped with a temperature control and stirring unit. Cuvettes with 10 mm path lengths were employed for all measurements. Ellipticity at 222 nm is reported as mean residue ellipticity [θ] (deg cm^2 dmol^{-1}) and was calibrated with (+)-10-camphorsulfonic acid. Samples were prepared by diluting an aqueous peptide stock solution into a buffer consisting of 1 mM each of sodium

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[1] Abbreviations: CD, circular dichroism spectroscopy; LEM, linear extrapolation method; GdmCl, guanidinium chloride; A, alanine; E, glutamic acid; K, lysine; Y, tyrosine; F, phenylalanine.

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phosphate, sodium borate, and sodium citrate (CD buffer) and the indicated amount of sodium chloride and denaturant. A solution of approximately 8 M urea or 6 M GdmCl in CD buffer was prepared fresh daily. The concentration of urea or GdmCl in these solutions was determined with refractive index measurements (Pace, 1986). In all cases, the pH was adjusted by the addition of HCl or NaOH to pH 7.0 at room temperature.

The concentration of the peptide stock solution was determined by ultraviolet absorbance of the single tyrosine chromophore using the extinction coefficient given by Brandts and Kaplan (1973). The effect of denaturant on the helicity of the peptides was determined by adding an aliquot of the concentrated denaturant solution in CD buffer to the sample of peptide in CD buffer and adjusting for volume changes after each addition. The reversibility of the transition was determined by diluting a sample of peptide in concentrated denaturant into CD buffer. In all cases the reversibility of the transition was ≥98%.

Use of the Zimm−Bragg Model to Quantify Helix Formation. The denaturant-induced unfolding curves were fitted by the Zimm−Bragg model for the helix−coil transition (Zimm & Bragg, 1959). To determine the equilibrium constant for helix formation (termed *s*-value), the observed mean residue ellipticity, $[\theta]_{obs}$, must be converted to fractional helicity, f_H, for each peptide at each denaturant concentration:

$$f_H = \frac{[\theta]_{obs} - [\theta]_C}{[\theta]_H - [\theta]_C} \quad (1)$$

where $[\theta]_H$ and $[\theta]_C$, the $[\theta]$ values for helix and coil, respectively, are defined as

$$[\theta]_H = H_0\left(1 - \frac{2.5}{n}\right) + H_D[\text{denaturant}] \quad (2)$$

$$[\theta]_C = C_0 + C_D[\text{denaturant}] \quad (3)$$

The coefficients of the last two terms in eqs 2 and 3, H_D and C_D, provide the denaturant-dependence of the ellipticities of the helix and coil, respectively, and the H_0 and C_0 terms are the ellipticities of the helix and coil at 0 °C in the absence of denaturant. A chain length dependence $(1 - 2.5/n)$ to H_0 is also included (Chen et al., 1974; Scholtz et al., 1991b). In this analysis, H_0 and H_D were varied or, alternatively, H_0 was fixed to −42 500 deg cm^2 dmol^{-1}, the value found in the previous report (Scholtz et al., 1995). Likewise, $[\theta]_C$, as defined by eq 3, could be determined from the least-squares analysis of the data or measured directly for the 7-residue, unstructured peptide, under each of the experimental conditions. The direct measure of $[\theta]_C$ for the 7-residue peptide is shown in Figure 1A for GdmCl and in the figures in the supporting information for all of the other denaturant combinations. For all of the data, the two methods for determining the base lines (eqs 2 and 3) gave similar estimates for cardinal parameters, s_0, m, or k (see below).

Circular dichroism data were fitted by the above equations using a version of the nonlinear least-squares procedure (Johnson & Frasier, 1985), which has been implemented for a Macintosh personal computer by Brenstein (1991). The best fit values for the variable parameters and their 67% confidence intervals (Johnson, 1983) were determined in fitting the data by the indicated model (see below).

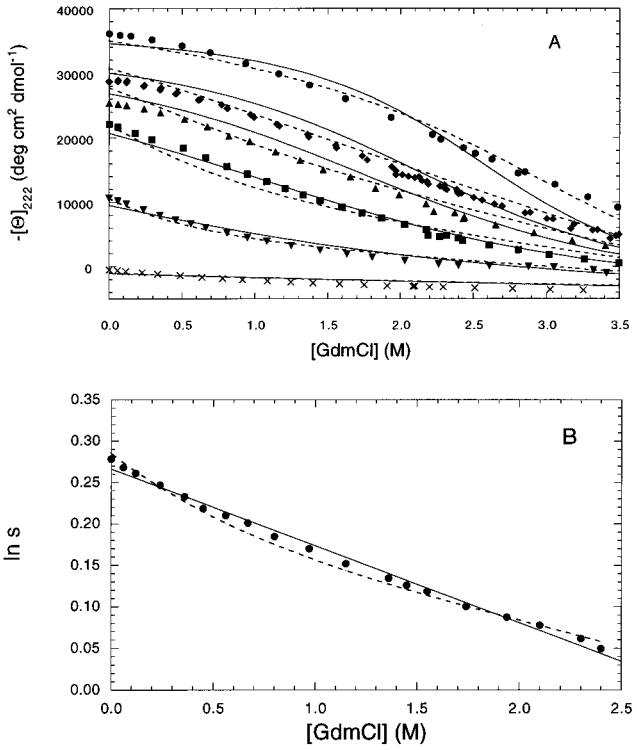

FIGURE 1: (A) Guanidine hydrochloride-induced helix to random coil transitions in the peptides as monitored by the change in the CD signal at 222 nm. The solution contained 1 M NaCl and 1 mM each of sodium phosphate, sodium citrate, and sodium borate at pH 7.0. The peptides vary in chain length but otherwise have the same general sequence: (●) 50; (◆) 32; (▲) 26; (■) 20; (▼) 14; (×) 7 residues. The solid lines are the best fit to the LEM, and the dashed lines are the fit to the thermodynamic binding model (see text for details). (B) A free energy plot of the best fit helix equilibrium constant (ln *s*) for the peptides shown in panel A at 1 M NaCl as a function of the molar concentration of guanidine hydrochloride. The solid line shows the linear fit to the data, corresponding to the LEM, and the dashed line is the fit of the data to the thermodynamic binding model (see text for details).

The observed fraction helix at any solution condition, f_H, calculated as shown in eq 1, is used to determine the Zimm−Bragg helix−coil transition theory parameters *s* and σ using

$$f_H = \frac{\sigma s}{(s-1)^3}\left(\frac{ns^{n+2} - (n+2)s^{n+1} + (n+2)s - n}{n\{1 + [\sigma s/(s-1)^2][s^{n+1} + n - (n+1)s]\}}\right) \quad (4)$$

where *n* is the number of amide groups in the peptide, *s* is the propagation parameter, and σ is the helix nucleation parameter. This equation is from the Zimm−Bragg model for helix formation (Zimm & Bragg, 1959) in which peptides are treated as homopolymers and only one helical stretch of residues is allowed in each chain. This model is identical to the full treatments of helix formation for peptides of these chain lengths [see Qian and Schellman (1992)]. Besides the propagation parameter, *s*, the other parameter that is required for the analysis in eq 4 is σ, the helix nucleation constant. The value of σ has been determined previously (Rohl et al., 1992; Scholtz et al., 1991b), and we find that 5-fold changes in σ do not affect either s_0, the denaturant dependence of *s*, or the quality of the overall fitting. Therefore, we hold σ constant in all fittings at the previously determined value of 0.003 (Rohl et al., 1992; Scholtz et al., 1991b).

Analysis of Denaturant-Induced Unfolding. There are two physical models that can be used to analyze urea- and guanidine-induced protein denaturation curves (Pace, 1986; Schellman, 1987, 1994). These include the thermodynamic binding or solvent exchange models, in which the interaction of the denaturant with the protein can be treated as specific binding (Aune & Tanford, 1969; Schellman, 1955, 1987) or a solvent exchange event, where the interaction of both the solvent (water) and the cosolvent (urea or GdmCl) with the protein is treated explicitly in an expression that involves the interchange between both components at a particular interaction "site" on the protein (Schellman, 1990). For the denaturation of helical peptides, where the thermodynamic unit is the peptide residue, these two models have the following general forms:

binding:

$$\ln s = \ln s_0 - \ln(1 + ka) \tag{5}$$

solvent exchange:

$$\ln s = \ln s_0 - \ln(A_1 + KA_3) \tag{6}$$

$$\ln s = \ln s_0 - [\ln f_1 + \ln(1 + (K' - 1)\chi_3] \tag{7}$$

where s_0 is the *s*-value for the homopolymeric residue in the absence of denaturant, k is the binding constant for denaturant to a residue, a is the molar activity of the denaturant solution, A_1 and A_3 are mole fraction activities of water and denaturant, f_1 is the mole fraction activity coefficient for water, χ_3 is the mole fraction of the denaturant solution, and K and K' are the exchange equilibrium constants for the indicated concentration scale. Denaturant activity is determined using the relationships between denaturant molarity and molar activity given by Pace (1986). The derivations of these models and the physical basis for their forms are given by Schellman (1987, 1990, 1994). The previous report (Scholtz et al., 1995) indicated that, for the case of these peptides, these two thermodynamic models are equivalent, and therefore, we will employ the simple binding model (eq 5) as one way to analyze our data.

An alternative method for quantifying denaturation curves for proteins is called the linear extrapolation method (LEM) (Greene & Pace, 1974; Santoro & Bolen, 1988, 1992). This empirical method expresses the Gibbs energy of folding as a linear function of denaturant molarity. For the helix to random coil transition, the LEM has the general form

$$\ln s = \ln s_0 - \frac{m[\text{denaturant}]}{RT} \tag{8}$$

where m is the change in the free energy of helix propagation per residue as a function of denaturant molarity, T is absolute temperature, $R = 1.987$ cal mol^{-1} K^{-1}, and the other terms have the same meanings as above. It has been shown that the physical models (eqs 5 and 6) for the effect of the denaturant−water system on protein or helix stability can be represented by the LEM equation under our conditions (Scholtz et al., 1995). Therefore, we will employ the LEM for our analysis; similar conclusions can be obtained by analyzing the data with the thermodynamic models (see below).

RESULTS

GdmCl Unfolding of Helical Peptides. Figure 1A shows the effects of adding aqueous GdmCl to the six peptides at a NaCl concentration of 1.0 M. An increase in the concentration of GdmCl causes a gradual change in the CD signal for these peptides, indicating a transition from peptides with stabilized helical segments at low [GdmCl] to those that approximate an unstructured random coil as indicated by the characteristics of the CD spectra at the extremes of either [GdmCl] or peptide chain length (CD spectra not shown). Since we have employed homologous peptides that only differ in chain length, we can make use of the homopolymer version of the Zimm−Bragg model for the helix to random coil transition (eq 4) and the expressions for the CD base lines (eqs 1−3), to find the dependence of *s*, the helix stability constant, on [GdmCl]. These results are shown in Figure 1B, where each data point represents the best fit *s*-value at the indicated GdmCl concentration using eqs 1 and 4 only, without any implicit form for the denaturant dependence on the *s*-value. The line in Figure 1B is the linear fit to the LEM (equation 8) and provides an *m*-value of 50 cal mol^{-1} M (GdmCl)$^{-1}$. Since the data in Figure 1B show some curvature, suggesting a deviation from the LEM, we have also analyzed the data with the binding model (eq 5). The dashed curve in Figure 1B is the best nonlinear fit to the data where $k = 0.2$, and the GdmCl concentration is expressed as molar activity (eq 5).

Although it appears that the binding model provides a better description for the effects of GdmCl on these peptides than the LEM, a second comparison of the two methods, provided by a global analysis of the raw data to eqs 1−4 and either 5 or 8 as shown by the curves in Figure 1A, affords nearly identical deviations (variance$^{1/2}$ = 1200 for LEM and 1630 for the binding model). Since the use of the LEM is widespread and given its simple form, we have elected to use the LEM to analyze the rest of our data. Also, since the curvature observed in Figure 1B, and hence the apparent deviation from the LEM, is dependent on the ionic strength of the solution (see below), it is not evident how best to treat this effect, so we will analyze these data with the simple LEM. It should be noted, however, that the conclusions that follow are still valid if we use the binding model instead of the LEM.

To explore the salt effects of GdmCl solutions on helix formation, we also performed two additional GdmCl-induced helix-unfolding experiments: one with 10 mM added NaCl and a second in which the total chloride concentration at each point was fixed at 3 M using combinations of NaCl and GdmCl. The results of these experiments, as well as a reproduction of the data from Figure 1B, are shown in the free energy plots in Figure 2. (The full plots of the raw data, in a form similar to that shown in Figure 1A, can be found in the supporting information.) The addition of NaCl decreases both the slope or *m*-value for GdmCl and the *y*-intercept, the free energy of helix formation in the absence of GdmCl.

Since the ionic strength of the solution is expected to alter the equilibrium between helix and random coil (Scholtz et al., 1991c), we investigated the effects of NaCl alone on helix formation. Figure 3 shows the free energy plots for the effects of NaCl on helix stability. These data can surprisingly be described with the LEM as well. Also shown in Figure 3 are three combinations of urea and NaCl. The first data set, where the urea concentration is varied in 0.1 M NaCl, is from a previous report (Scholtz et al., 1995). The second experiment shows the effects of increasing concentrations

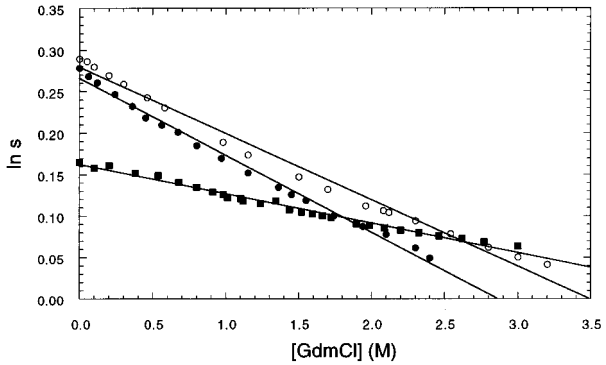

FIGURE 2: Free energy plots for helix formation as a function of guanidine hydrochloride concentration at different concentrations of NaCl: (○) 0.01 M; (●) 1.0 M; (■) total $Cl^- = 3.0$ M. The solid lines are the results of the analysis of each set of data with the LEM for the effects of GdmCl on the helix to random coil transition.

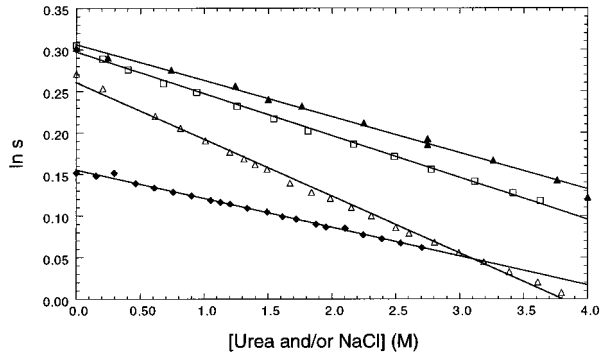

FIGURE 3: Free energy plots for helix formation as a function of urea and/or NaCl concentration: (▲) variable NaCl and no urea; (□) variable urea in 0.1 M NaCl; (△) variable urea and NaCl such that [urea] = [NaCl] at every point; (◆) variable urea in 3.0 M NaCl. The solid lines are the results of the analysis of each set of data with the LEM for the effects of urea or NaCl on the helix to random coil transition.

of urea in the presence of a fixed concentration of NaCl (3.0 M). Finally, the concentrations of urea and NaCl were varied simultaneously such that the urea concentration is exactly the same as the concentration of NaCl at each point. All of the data used to construct Figures 2 and 3 are available as supporting information, with the exception of the urea data in 0.1 M NaCl, which is described in detail in a previous report (Scholtz et al., 1995).

The results of the analysis of the seven free energy plots are collected in Table 1. These results represent the independent analysis of each of the seven data sets using the LEM to provide measures of the *m*-value or slope of the free energy plot and ln s_0, the helix equilibrium constant in the absence of denaturant or salt. The *m*-values for GdmCl (first three entries) are strongly dependent on the total ionic strength of the solution as expected since GdmCl is an ionic denaturant. However, the *m*-values for urea (last three entries) also show a rather strong dependence on the ionic strength of the solution. The ionic strength of the solution also affects ln s_0 for all denaturants employed. In the following section we attempt to rationalize these results and separate the denaturant effects of these solutions from the general salt effects on helix formation.

DISCUSSION

A common method employed to determine the conformational stability of a protein is through analysis of solvent−denaturation curves, employing urea or GdmCl as the chemical denaturant. Like proteins, the structure in helical peptides is also affected by aqueous solutions of GdmCl and urea. Unlike proteins, however, the helix to random coil transition in peptides cannot be treated as a simple two-state reaction between completely helical molecules and the unstructured peptide. Fortunately, the helix to random coil transition can be described adequately by a number of models, including those of Zimm and Bragg (1959) and Lifson and Roig (1961). In these models, the equilibrium constant for propagation (*s*- or *w*-value, respectively, for the two models), represents a two-state helix−coil transition for each residue in the peptide. Therefore, ln *s* from helix−coil theory is analogous to ln K_{eq} from the two-state model commonly used to analyze protein-unfolding transitions. In these studies, we make use of this simple relationship and can therefore investigate the effects of denaturants on helix propagation. In a previous report (Scholtz et al., 1995), we investigated the effects of urea on the helix−coil transition in these peptides. Here we extend the studies to include the other popular protein denaturant GdmCl. We also have investigated the effects of a neutral salt, NaCl, and combinations of urea and NaCl or GdmCl and NaCl in an attempt to separate the denaturant effects of GdmCl solutions from the general ionic strength effects.

Table 1: Analysis of the Individual Helix-Unfolding Transitions

conditions[a]	ln s_0 [b]	*m*-value[c]
variable GdmCl in 0.01 M NaCl	0.279	43.4
variable GdmCl in 1.0 M NaCl	0.266	50.4
variable GdmCl + NaCl with $[Cl^-] = 3.0$ M	0.165	20.0
variable NaCl	0.297	27.4
variable urea in 0.10 M NaCl[d]	0.306	23.7
variable urea in 3.0 M NaCl	0.155	18.8
variable urea + NaCl with [urea] = [NaCl]	0.260	37.1

[a] Each experiment was performed at 0 °C in a buffer that contained 1 mM each of sodium phosphate, sodium citrate, and sodium borate at pH 7.0 with the indicated amount of NaCl or denaturant. [b] Calculated from the *y*-intercept of the free energy plots (Figures 2 and 3) using eq 8. The error ranges from 0.005 to 0.009. [c] Calculated from the slope of the free energy plots (Figures 2 and 3) using eq 8. The error ranges from 0.4 to 0.7 cal mol^{-1} M^{-1}. These *m*-values are expressed *per residue*, in cal mol^{-1} M^{-1}. [d] Data from a previous report (Scholtz et al., 1995).

The seven different combinations of urea, GdmCl and NaCl employed are summarized in Table 1. In the results presented in Table 1, each free energy plot of the type shown in Figures 2 and 3 has been analyzed individually with the LEM (eq 8). We have also performed an analysis of the data represented by Figures 2 and 3, separating the molar concentrations of the components into two species: total chloride, from either GdmCl or NaCl, and total denaturant, either urea or the guanidinium cation. A simple expression based on the LEM was then fitted simultaneously to the seven data sets.

$$\ln s = \ln s_0 - \frac{m_{Cl}[Cl^-]}{RT} - \frac{m_{den}[\text{denaturant}]}{RT} \qquad (9)$$

with ln s_0, m_{Cl} and m_{den} as variable parameters. The best fit values for these parameters and their 67% confidence limits are shown in Table 2. The lines in Figure 4 show the results of this analysis for the seven sets of experimental conditions with the data points reproduced from Figures 2 and 3 for

Table 2: Global Analysis of the Helix-Unfolding Transitions

	best fit values[a]	
parameter[b]	single denaturant term[c]	separate denaturant terms[d]
ln s_0	0.286 ± 0.006	0.286 ± 0.007
m_{Cl}	20.4 ± 1.0 cal mol^{-1} M^{-1}	20.4 ± 1.3 cal mol^{-1} M^{-1}
m_{den}	23.4 ± 1.4 cal mol^{-1} M^{-1}	
m_{urea}		23.5 ± 1.3 cal mol^{-1} M^{-1}
m_{Gdm^+}		23.3 ± 1.5 cal mol^{-1} M^{-1}
(variance)$^{1/2}$	0.0107	0.0107

[a] The best fit value for each parameter is given along with the 67% confidence interval. [b] Parameters used in the analysis of the data using either eq 9 or 10. The square root of the variance of the overall fit is given as the last entry. [c] Three-parameter nonlinear least-squares fit of eq 9 to the data shown in Figures 2 and 3. The denaturant effects of urea and Gdm^+ are treated as a single term. [d] Four-parameter nonlinear least-squares fit of eq 10 to the data shown in Figures 2 and 3. The denaturant effects of urea and Gdm^+ are treated as separate terms.

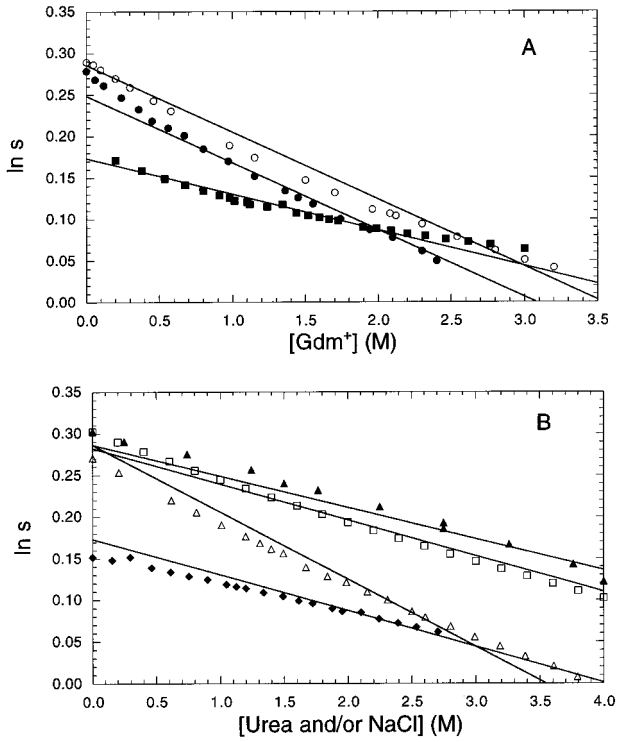

FIGURE 4: (A) Free energy plots for helix formation as a function of guanidine hydrochloride concentration at different concentrations of NaCl: (○) 0.01 M; (●) 1.0 M; (■) total Cl^- = 3.0 M. The solid lines are the results of the global analysis of all available data using eq 9 for the independent effects of Gdm^+ or urea and Cl^- on the helix−random coil transition. (B) Free energy plots for helix formation as a function of urea and/or NaCl concentration: (▲) variable NaCl and no urea; (□) variable urea in 0.1 M NaCl; (△) variable urea and NaCl such that [urea] = [NaCl] at every point; (◆) variable urea in 3.0 M NaCl. The solid lines are the results of the global analysis of all available data using eq 9 for the independent effects of Gdm^+ or urea and Cl^- on the helix to random coil transition.

comparison. From these results, two important points can be made: (1) for the helix to random coil transition in these peptides, it is possible to separate the denaturant effects of aqueous guanidine hydrochloride solutions into two components, an ionic component which is proportional to the total molar concentration of chloride and a denaturant component which is linear in the molar concentration of the guanidinium cation (Gdm^+) and (2) the denaturant strength, as manifested in the *m*-value for urea and Gdm^+, on a molar basis, are equivalent.

It is also possible to treat the denaturant effects of urea and Gdm^+ as separate terms (eq 10). When this approach is used, we find that the *m*-values for urea and Gdm^+ are identical, within error (Table 2) to those found when we group the denaturant effects into a single term.

$$\ln s = \ln s_0 - \frac{m_{Cl}[Cl^-]}{RT} - \frac{m_{urea}[urea]}{RT} - \frac{m_{Gdm^+}[Gdm^+]}{RT} \quad (10)$$

Furthermore, the ln s_0 values are identical and, more importantly, the square root of the variance of the fit does not change when we use separate terms for urea and Gdm^+. This is strong evidence for the validity of our conclusion regarding the equivalence of urea and Gdm^+ as perturbants of helix formation in these peptides.

How can we relate these results to protein studies? There are several features of the helix to random coil transition in these peptides that are unique and may not apply to protein unfolding reactions. First, the amount of helical structure, and the stability of that structure, is quite sensitive to the ionic strength of the solution (Marqusee & Baldwin, 1987; Scholtz et al., 1991c, 1993). Most proteins do not show this strong ionic strength dependence on stability (or structure) for "neutral" salts, such as NaCl. Second, the overall stability of the helical form of these peptides is very small when compared to the conformational stability of even small globular proteins (Pace, 1975, 1990), presumably due in part to the lack of a well-developed hydrophobic core and other tertiary interactions in the helix.

For most small proteins, GdmCl is found to be approximately 2.3 times as effective as a denaturant than urea, on the basis of the ratio of *m*-values (Myers et al., 1995). Amino acid solubility studies (Nozaki & Tanford, 1963, 1970) also find that GdmCl and urea are both good solubilizing agents for amino acids, with GdmCl being 2−3 times more effective than urea at the same concentration. The results for helix unfolding support this finding (Table 1); under some conditions, however, we find that the effectiveness of GdmCl and urea as purturbants depends strongly on the ionic strength of the solution. In fact, an equimolar mixture of urea and NaCl is almost as effective as a helix-unfolding agent as a solution of GdmCl. To our knowledge, solubility studies on amino acids or model compounds in urea as a function of total ionic strength (e.g., with NaCl) have not been performed.

One major difference between the helix-unfolding reaction, compared with denaturant-induced unfolding of proteins, is the large relative contribution that the peptide backbone may play in the transition. For proteins, the contributions of the change in accessible surface area upon unfolding for nonpolar and polar groups are both important. In helix unfolding, however, the exposure of the peptide backbone is the largest factor, since the change in solvent accessible surface for the side chains is rather small, especially compared to protein unfolding, while the backbone, including the peptide group, undergoes a large change in accessibility to solvent. For this reason, it is instructive to compare the effects of urea, NaCl, and GdmCl on the solubility or transfer free energy of the peptide group with effects of these agents on side-chain analogs. There have been several reports that have addressed this point using a variety of model compounds (Nozaki & Tanford 1963, 1970; Nandi & Robinson, 1984;

Liu & Bolen, 1995). Although different model compounds give slightly different quantitative estimates of the transfer free energy for the peptide group from water to aqueous solutions of urea or GdmCl, the studies all find that the transfer is favorable; that is, the peptide group is more soluble in an aqueous denaturant solution that in pure water [summarized in Table 4 of Liu and Bolen (1995)]. For a non-denaturing salt, such as NaCl, Nandi and Robinson (1974a) found that the transfer from water is also favorable. Therefore, the peptide group is more soluble in solutions of urea, GdmCl, or NaCl than in pure water, and thus these agents promote the helix-unfolding reaction. In contrast, the side chains of the amino acid residues show a wide variability in transfer free energy or solubility in urea and GdmCl solutions (Nozaki & Tanford, 1963; 1970). However, at least for the nonpolar residues, the transfer free energy to aqueous NaCl solutions is unfavorable (Nandi & Robinson, 1974b). Thus, protein unfolding by ionic agents is a balance between favorable and unfavorable transfer events, while the helix-unfolding transition is favored with all three agents investigated here.

There are several reports of GdmCl and urea providing different estimates for the conformational stability of a protein [Pace, 1975; but see Yao and Bolen (1995) for a possible explanation]. In some cases, these differences have been attributed to the ionic nature of GdmCl. In a striking example, Monera and co-workers have investigated the effects of urea and GdmCl as denaturants of a synthetic coiled-coil peptide, containing variable numbers of inter- and intrahelical electrostatic interactions (Monera et al., 1994). They found that analysis of urea- and GdmCl-denaturation curves provided different estimates of the stability of their peptides and that GdmCl masks electrostatic interactions. Our results are consistent with this interpretation and furthermore we conclude that almost half of the helix denaturant potential of aqueous GdmCl may be due to general salt effects on the peptide group. Further work is underway to test this result in other peptides, with other salts, and for protein denaturation.

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SUPPORTING INFORMATION AVAILABLE

Five figures (similar to Figure 1A) illustrating the effects of GdmCl, urea, and/or NaCl on the CD signal at 222 nm for the peptides (6 pages). Ordering information is given on any current masthead page.

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