Residue Helix Parameters Obtained from Dichroic Analysis of Peptides of Defined Sequence†

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ABSTRACT: Circular dichroic measurements of the host peptide acetyl-Y(EAAAK)$_3$A-amide were obtained in solutions of increasing ionic strength at pH 7.0 and 0 °C. The changes observed in the dichroic spectra are characteristic for a two-state helix/coil transition. The mean residue ellipticity at 222 nm exhibits a curvilinear dependence on ionic strength which becomes linear at ionic strengths greater than 1 M. The slope of the linear portion is assumed to represent the lyotropic character of the salt, and its extrapolated intercept is assumed to represent the mean residue ellipticity of the peptide solution freed from both electrostatic and lyotropic contributions which affect the helical stability of the host peptide. An extrapolated mean residue ellipticity value was obtained for each host peptide having a different amino acid guest residue at position 9 in the peptide sequence. These values were used to calculate a propagation parameter, s, for each residue using the Lifson–Roig algorithm for peptide helical content and assuming a common nucleation parameter of 0.003. The ability of these minimally determined residue parameters to predict the helical content of a variety of peptides is encouraging. Estimates were also made of the ΔG values for the electrostatic interactions within the host peptide and for the additional interactions generated by ionic guest residues.

A variety of investigative groups (Lyu et al., 1990; Merutka et al., 1990; O'Neil & DeGrado, 1990; Padmanabhan et al., 1990; Chakrabartty et al., 1991; Gans et al., 1991; Stellwagen et al., 1992; Chakrabartty & Baldwin, 1993) have analyzed the helical content of host/guest peptides of defined sequence. The majority of these efforts have relied on circular dichroic measurements of mean residue ellipticity which are interpreted in terms of a two-state helix/coil transition. Guest residues have been ranked in terms of their relative ability to stabilize the helical conformation. Estimates of the helix propagation parameter, s, for some of the residues have been reported using the Lifson–Roig algorithm for estimation of the helical content of peptides of defined sequence and modest length.

In this report, we utilize the peptide acetyl-Y(EAAAK)$_3$A-amide as a host peptide for determination of an s value for each of the 19 common guest amino acid residues at position 9 in the center of the host sequence. Dichroic measurements were made in the presence of increasing concentrations of salt in an effort to eliminate electrostatic and lyotropic contributions to the measurement of peptide helix content.

EXPERIMENTAL PROCEDURES

Materials. All peptides were synthesized from tBOC amino acids by the simultaneous multiple peptide synthetic procedure described by Houghten et al. (1986). The peptide preparations were purified by reversed-phase chromatography using a 21.5 × 250 mm Hamilton PRP-1 preparative column and an Isco gradient chromatograph. Each peptide preparation was partitioned in a linear gradient between 14% and 24% acetonitrile in 0.1% trifluoroacetic acid which was generated in 20 min using a flow rate of 8 mL/min at ambient temperature. The column effluent was monitored at 220 nm, and fractions containing 4 mL were collected.

Selected fractions were concentrated by lyophilization and subjected to analytical chromatography, to amino acid compositional analysis, and to FAB mass spectrometry. Analytical reversed-phase chromatography was performed at ambient temperature using a 4.1 × 150 mm Hamilton PRP-1 analytical column and a linear gradient between 14% and 24% acetonitrile in 0.1% trifluoroacetic acid which was generated in 20 min using a flow rate of 1 mL/min. The elution profile observed for each peptide preparation is characterized by a single peak whose area accounts for at least 98% of the material eluted from the column. The amino acid compositional analyses had the following mean molar ratios with the standard deviation given in parentheses: A/E 3.03 (0.06); K/E 1.00 (0.01); guest/Y 1.00 (0.05). The mass/charge ratio of the main molecular ion of each peptide was within 0.6 mass unit of that expected for the singly protonated peptide.

The concentration of each peptide solution was calculated from its alkaline difference absorbance spectrum observed at 243 ± 1 nm between pH values of 6.5–7.0 and 12.5–13.0, using a difference extinction coefficient of 11.1 mm^{-1} cm^{-1}.

Dichroic Measurements. All circular dichroic measurements were made using an Aviv Model 62DS spectrometer equipped with a thermoelectric temperature controller. The instrument was routinely adjusted at ambient temperature such that a 1 mg/mL solution of *d*-10-camphorsulfonic acid had an ellipticity of 335 mdeg at 290 nm in a cell with a 10-mm optical path and had a ratio of ellipticities at 192.5 and at 290 nm of −2.00 ± 0.01 in a cell with a 1-mm optical path. All peptide measurements were made at 0 °C in solutions containing 20–450 μM peptide, 1 mM phosphate buffer, and the indicated concentration of KCl or $CaCl_2$ using rectangular cells with 0.1–10-mm optical paths. All ellipticity measurements are reported as mean residue ellipticity, $[\theta]$, having the units degrees centimeter squared per decimole of residues. Dichroic measurements of different solutions of the same

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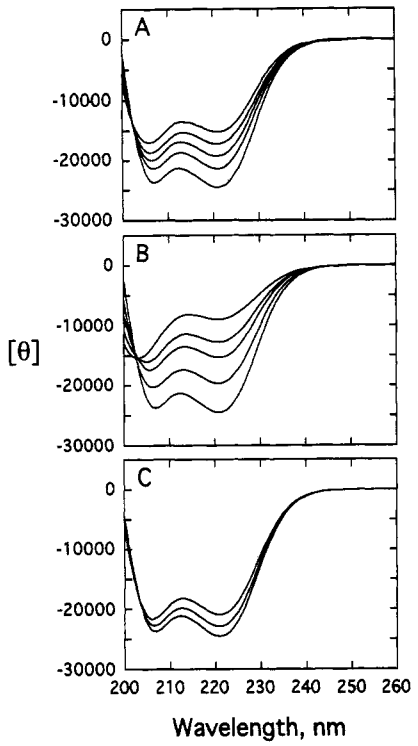

FIGURE 1: Circular dichroic spectra of the host peptide. Spectra obtained at pH 7.0 and 0 °C in the presence of added KCl and added $CaCl_2$ are illustrated in panels A and B, respectively. These illustrated spectra were obtained, reading upward at 220 nm, with solutions having ionic strengths of 0.0, 0.5, 1.5, 2.5, and 3.5. Spectra obtained in the absence of salt at pH values ranging from 2.0 to 9.0 are illustrated in panel C. The uppermost spectrum was obtained at pH 2.0 and 2.8, the intermediate spectrum at pH 4.4, and the lowermost spectrum at pH 6.7 and 9.0.

peptide obtained over the course of this study typically gave a variation in mean residue ellipticity of ±500 deg cm^2 $dmol^{-1}$ which corresponds to a variation in ΔG of no more than ±0.05 kcal/mol.

pH Measurements. All pH measurements were made using a Radiometer Model PHM 82 pH meter and a GK 473901 combined pH electrode. The instrument was calibrated just prior to use with standard buffers having pH values of 4.01, 7.12, and 10.23 at 0 °C.

Residue Helical Parameters. A program was written to calculate Zimm–Bragg σ and s values for prediction of the helical content of a peptide from its sequence. This program is based on the Lifson–Roig algorithm (1961) as modified by Qian and Schellman (1992). The program is designed to simultaneously fit up to 15 peptides of known sequence and helix content to a common set of residue helix nucleation and propagation parameters, σ and s, respectively. Fitting was done using the algorithm of Brent (1973) for minimization of χ^2 differences between the calculated and observed helical contents. Conversely, the program can be used to predict the helical content of any peptide sequence containing fewer than 60 residues given a library of σ and s values for each residue.

RESULTS

Measurements at pH 7.0. The response of the circular dichroic spectrum of the host peptide to the addition of KCl or $CaCl_2$ at pH 7.0 is illustrated in Figure 1, panels A and B. The addition of these salts produces a nested series of spectra having an isodichroic point at 203 nm and a mean residue ellipticity of −16 300 ± 1000 deg cm^2 $dmol^{-1}$. This isodichroic point together with the accompanying changes in

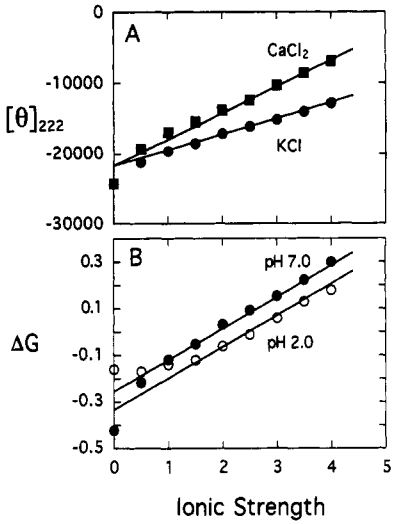

FIGURE 2: Ionic strength dependence of the host peptide. Panel A illustrates the mean residue ellipticity of the host peptide at 222 nm observed at pH 7.0 and 0 °C in the presence of increasing concentrations of KCl (circles) and of $CaCl_2$ (squares). Panel B compares the ΔG values for the host peptide in increasing concentrations of KCl at pH 7.0 (filled circles) and at pH 2.0 (open circles). All values were calculated from ellipticity measurements at 222 nm using eq 1.

the spectra is characteristic for a two-state transition involving the α-helical and coil conformations of a peptide in solution. The ΔG for helix formation in a two-state helix/coil transition can be calculated from an observed mean residue ellipticity at 222 nm using eq 1. The 17-residue host peptide was assumed

$$\Delta G = -RT \ln \frac{[\text{helix}]}{[\text{coil}]} = -RT \ln \frac{[\theta]_{\text{observed}} - [\theta]_{\text{coil}}}{[\theta]_{\text{helix}} - [\theta]_{\text{observed}}} \quad (1)$$

to have mean residue ellipticity values at 222 nm of −35 600 and +300 deg cm^2 $dmol^{-1}$ in the α-helical and in the coil conformations, respectively (Merutka et al., 1990), unless noted otherwise.

The mean residue ellipticity of the host peptide measured in the absence of added salt at pH 7.0 and 0 °C is −24 300 deg cm^2 $dmol^{-1}$ at 222 nm, as shown in Figure 2A. This observed ellipticity corresponds to a ΔG of −0.42 kcal/mol, as shown in Figure 2B. This measured value, denoted as ΔG_m, likely includes electrostatic contributions to the helix/coil equilibrium of the host peptide. The addition of the salts KCl or $CaCl_2$ to solutions of the host peptide decreases the negative value of its mean residue ellipticity, as shown in Figure 2A. The ellipticity exhibits a linear dependence on ionic strength above 0.5 M. The slope of the linear dependence is different in solutions containing KCl and $CaCl_2$ as shown in Figure 2A. The slope associated with each salt is assumed to reflect its lyotropic contribution to the helix/coil equilibrium of the host peptide. The linear dependence observed with each salt extrapolates to a common intercept of −21 700 deg cm^2 $dmol^{-1}$, as shown in Figure 2A. This value corresponds to a ΔG of −0.25 kcal/mol, as shown in Figure 2B. This extrapolated value, denoted as ΔG_e, is assumed to represent the helix/coil equilibrium of the host peptide free from electrostatic interactions and free from the lyotropic contribution of the particular salt employed. The difference between the measured and extrapolated ΔG values for the host peptide, denoted as $\Delta\Delta G_{m-e}$, is −0.17 kcal/mol. This difference is assumed to represent the contribution of electrostatic interactions to the stabilization of the helical conformation of the host peptide at pH 7.0 and 0 °C.

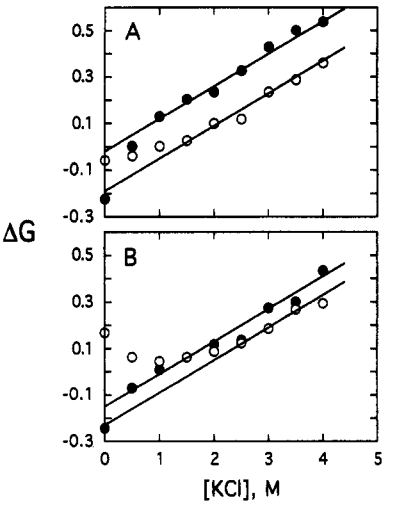

FIGURE 3: Ionic strength dependence of an acidic and a basic guest residue. The measurements shown in panel A were obtained using the peptide having a glutamate guest residue and in panel B for the peptide having a lysine guest residue. The filled circles indicate measurements at pH 7.0 and 0 °C, and the open circles indicate measurements at pH 2.0 and 0 °C.

Table I: ΔG Values for Host/Guest Peptides at 0 °C[a]

guest residue	pH 7			pH 2			pH 2–7	
	ΔG_m	ΔG_e	$\Delta\Delta G_{m-e}$	ΔG_m	ΔG_e	$\Delta\Delta G_{m-e}$	$\Delta\Delta G_m$	$\Delta\Delta G_e$
A	–0.42	–0.25	–0.17	–0.16	–0.38	0.22	0.26	–0.13
C	–0.06	0.14	–0.20	0.26	0.07	0.19	0.32	–0.07
D	0.18	0.35	–0.17	0.33	0.16	0.17	0.15	–0.19
E	–0.22	–0.02	–0.20	–0.06	–0.19	0.13	0.16	–0.17
F	–0.24	–0.05	–0.19	0.03	–0.19	0.22	0.27	–0.15
G	0.59	0.79	–0.20	0.87	0.69	0.18	0.28	–0.10
H	0.22	0.31	–0.09[b]	0.66	0.26	0.40	0.44	–0.05
I	–0.02	0.16	–0.18	0.20	–0.02	0.22	0.22	–0.18
K	–0.24	–0.14	–0.10	0.17	–0.23	0.41	0.41	–0.09
L	–0.27	–0.10	–0.17	0.03	–0.21	0.24	0.30	–0.11
M	–0.21	–0.04	–0.17	0.07	–0.13	0.20	0.28	–0.09
N	0.24	0.37	–0.13	0.49	0.26	0.23	0.25	–0.11
Q	–0.17	0.06	–0.23	0.09	–0.06	0.15	0.26	–0.12
R	–0.39	–0.29	–0.10	–0.02	–0.42	0.44	0.37	–0.13
S	0.10	0.27	–0.17	0.39	0.17	0.22	0.29	–0.10
T	0.24	0.44	–0.20	0.53	0.35	0.18	0.29	–0.09
V	0.24	0.45	–0.21	0.52	0.28	0.24	0.28	–0.17
W	–0.12	0.04	–0.16	0.06	–0.07	0.13	0.18	–0.11
Y	–0.07	0.13	–0.20	0.19	0.03	0.16	0.26	–0.10

[a] The values for helix formation in the absence of salt, ΔG_m and ΔG_e, were obtained at 0 °C by direct measurement and by extrapolation from salt-containing solutions, respectively, and have the units kilocalories per mole. [b] Measurements of the protonated form of the histidine guest residue were done at pH 5.6.

The effect of added KCl on the observed mean residue ellipticity of each host/guest peptide at pH 7.0 and 0 °C was analyzed in the same manner. The dependence of the ΔG for a representative acidic guest residue and a representative basic guest residue is illustrated in Figure 3. The linear dependence of ΔG on the [KCl] of the host/guest peptides solutions has a mean slope of 0.12 kcal/mol per M KCl with a standard deviation of 0.02. The ΔG_m, ΔG_e, and $\Delta\Delta G_{m-e}$ values for each host/guest peptide measured at pH 7.0 and 0 °C are listed in Table I. The mean $\Delta\Delta G_{m-e}$ values for the host peptides containing neutral, acidic, and basic guest residues are compared in Table II. These mean values are within the variance of a given measurement, ±0.05 kcal/mol, suggesting that the presence of an ionic guest residue does not significantly perturb the electrostatic stabilization of the helical conformation of the host/guest peptides at pH 7.0.

Measurements at Variable pH Values. The circular dichroic spectra shown in Figure 1C indicate that the fractional helical content of a solution of the host peptide is diminished by stepwise acidification in the absence of added salt. The appearance of an isodichroic point at 203 nm suggests that these pH-dependent changes can also be considered in terms of a two-state helix/coil transition using eq 1. The pH dependence of the ΔG_m for the host peptide measured in the absence of added salt is shown in Figure 4A. This dependence can be fit with a two-state helix/coil transition having an apparent pK of 4.2 and $\Delta\Delta G_m$(pH 2–7) of 0.26 kcal/mol. This apparent pK is appropriate for the protonation of the glutamate residues in the host peptide. Such protonation would eliminate the glutamate/lysine and the glutamate/N-terminal frayed end electrostatic interactions which contribute to the stabilization of the peptide helix.

Table II: Mean $\Delta\Delta G$ Values by Guest Residue Type[a]

guest residue type	$\Delta\Delta G_{m-e}$		pH 2–7	
	at pH 7	at pH 2	$\Delta\Delta G_m$	$\Delta\Delta G_e$
neutral	–0.19 (0.03)	0.20 (0.03)	0.27 (0.03)	–0.12 (0.03)
acidic	–0.19 (0.02)	0.15 (0.02)	0.15 (0.01)	–0.18 (0.01)
basic	–0.10 (0.01)	0.40 (0.02)	0.41 (0.04)	–0.09 (0.04)

[a] The individual ΔG values used in these comparisons are listed in Table I. The acidic residues are E and D; the basic residue are H (at pH 5.6 instead of 7.0), K, and R; the neutral residues are all the remaining common residues except for P. The first number listed in each column is the mean value, and the second number, which is in parentheses, is the standard deviation. All values have the units kilocalorie per mole at 0 °C.

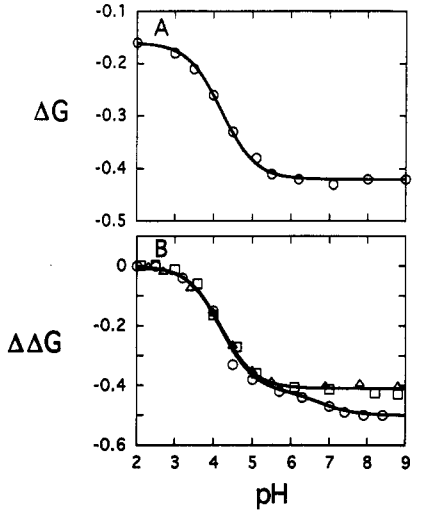

FIGURE 4: pH dependence. All measurements were obtained at 0 °C in the absence of added salt. Panel A illustrates measurements of the host peptide. Panel B illustrates measurements of the guest peptides containing histidine (circles), lysine (triangles), and arginine (squares). The observed ΔG values for these peptides are presented as $\Delta\Delta G_m$ values relative to the ΔG measured at pH 2.

The effect of acidification on the ΔG_m for each of the host/guest peptides has been measured in the absence of added salt. The pH dependence of the ΔG_m observed for each peptide can be fit with a two-state helix/coil transition having an apparent pK of 4.2 ± 0.1 and the $\Delta\Delta G_m$(pH 2–7) values listed in Table I. The mean $\Delta\Delta G_m$(pH 2–7) values for neutral, acidic, and basic guest residues are compared in Table II. The mean value for the basic guest residues appears to be significantly different.

The ΔG_m for the host peptide containing a histidine guest residue exhibits a unique dependence in the neutral pH range as shown in Figure 4B. This dependence can be fit with a two-state helix/coil transition having an apparent pK of 6.7 and a $\Delta\Delta G_m$(pH 5.6–8.5) of 0.09 kcal/mol. This apparent

pK is appropriate for the imidazole side chain of the guest histidine residue. Protonation of the histidine guest residue thus appears to modestly diminish the helical content of its host/guest peptide.

Measurements at pH 2.0. The mean residue ellipticity of the host peptide measured at pH 2.0 and 0 °C in the absence of added salt corresponds to a ΔG_m of –0.16 kcal/mol. This value appears to be insensitive to added KCl until a concentration of about 1.5 M is exceeded, as indicated by the open circles in Figure 2B. Further addition of KCl generates a linear increase in ΔG, having a slope of 0.14 kcal/mol per M KCl and an intercept, ΔG_e, of –0.38 kcal/mol. This slope is identical with that observed at pH 7.0, consistent with the view that it represents the lytotropic contribution of the salt to the helix/coil equilibrium of the host peptide. In contrast to the $\Delta\Delta G_{m-e}$ at pH 7.0, the $\Delta\Delta G_{m-e}$ at pH 2.0 is positive, having a value of 0.22 kcal/mol. This positive value indicates that the salt masks an antagonistic electrostatic interaction which diminishes the helical content of the host peptide in the absence of salt.

The only ionic residues in the host peptide at pH 2.0 are three lysine residues. Their $i,i+5$ spacing would not be anticipated to generate much electrostatic antagonism in the helical conformation. A more likely antagonistic electrostatic interaction would involve the positively charged N-terminal helical frayed end. At pH 7.0, this terminal charge is complemented by the anionic glutamate residue at position 2. Protonation of this glutamate would eliminate the complementary charged residue, leaving an unpaired N-terminal charge in the helical conformation which would diminish the fractional helical concentration of the peptide. However, addition of mobile anions to the solvent should restore complementation and enhance the helical content of the host peptide. It is noteworthy that full achievement of this complementation requires solvents of rather high ionic strength.

$\Delta\Delta G_{m-e}$ observed for each host/guest peptide at pH 2.0 has a positive value as shown in Table I. This observation is consistent with the proposed role of the positively charged N-terminal helical frayed end in these measurements. However, the mean $\Delta\Delta G_{m-e}$ value observed for the basic guest residues is significantly greater than that observed for either the neutral or the acidic guest residues at pH 2.0, as indicated in Table II. This difference can be clearly seen by comparison of the measurements presented in Figures 2B and 3. The larger $\Delta\Delta G_{m-e}$ observed for host peptides with basic guest residues likely results from an $i,i+3$ antagonistic electrostatic interaction in the helical conformation between the basic host lysine residue at position 6 and a basic guest residue at position 9.

As seen in Figures 2B and 3, the ΔG_e values observed for a given host/guest peptide are not the same at pH 2.0 and 7.0. The $\Delta\Delta G_e$(pH 2–7) values listed for each host/guest peptide in Table I have a mean value of –0.12 kcal/mol with a standard deviation of 0.03. This persistence likely reflects the difference in a property of the protonated and unprotonated forms of the host glutamate residues. The mean residue ellipticity of the tripeptide acetylYEAamide at 222 nm is independent of pH over the range pH 2.0–7.0 within the precision of measurement. This persistence indicates that the mean residue ellipticities of the two forms of a glutamate residue are equivalent at 222 nm. It would appear that the protonated glutamate residues have a greater helical preference than the unprotonated glutamate residues as suggested in the analysis of host/guest peptides of undefined sequence (Wojcik et al., 1990).

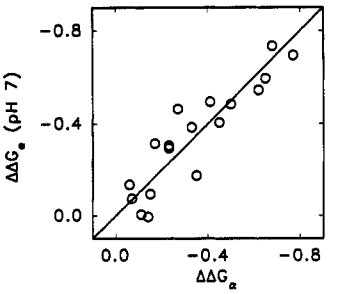

FIGURE 5: Comparisons with prior measurements. The abscissa is a measure of the relative helix stability of the host peptide acetylEWEALEKKLAALEXKLQALEKKLEALEHG whose guest residue X is exposed in the helical dimer (O'Neil & DeGrado, 1990). The ΔG for the helix dimer formation of each host/guest peptide was measured in 5 M urea at 23 °C. The values for the ionic residues were obtained either by measurement or by extrapolation in solvents containing 1.0 M NaCl. The ΔG observed for glycine host/guest peptide was subtracted from all the remaining measurements to generate the displayed $\Delta\Delta G_\alpha$ values having the units kilocalories per mole. The ordinate is a measure of the relative helix stability of the host/guest peptides used in this study; 0.35 kcal/mol has been subtracted from each ΔG_e value at pH 7 listed in Table I to normalize the span of the two scales. The value for the glycine guest residue, not shown, has the coordinates 0.00, –0.44.

DISCUSSION

Helix Parameters. As illustrated in Table I, the ΔG_e for helix formation of the host peptide acetyl-Y(EAAAK)$_3$A-amide at pH 7.0 and 0 °C ranges from –0.29 to 0.79 kcal/mol as the guest residue is varied. This range indicates that the identity of a single guest residue in the central region of a helical peptide can exert a profound effect on its helix/coil equilibrium. The ΔG_e values for individual guest residues in this host peptide compare favorably with the $\Delta\Delta G_\alpha$ values reported by O'Neil and DeGrado (1990) for the effect of guest residues on the stability of a dimeric helical host peptide, as shown in Figure 5. It should be noted that the sequence of the host peptide and the analysis of helical stability differ considerably in these two studies. Observation of a similar dependence of guest residues on helix stability suggests that this relationship is not sequence or analysis limited.

If the ΔG_e values measured here have any global relevance, it should be possible to predict the helical content of unrelated peptides measured using the same extrapolation protocol. This possibility has been explored using the Lifson–Roig (1961) statistical mechanical model for prediction of peptide helical content. Use of this model requires the peptide sequence and two parameters for each residue, a nucleation parameter and a propagation parameter. These parameters have been related (Qian & Schellman, 1992) to the corresponding parameters in the Zimm–Bragg model (1959), σ and s, respectively. In accordance with common practice, σ is assumed to be 0.003 for each residue (Scholtz & Baldwin, 1992).

The Lifson–Roig algorithm was first used to obtain an s value for each guest residue at pH 7.0 and 0 °C from the measured percent helical content of each host/guest peptide. The percent helical content was calculated for each peptide from its extrapolated mean residue ellipticity, obtained as illustrated in Figure 2A, and the mean residue ellipticity of a helix and of a coil. The mean residue ellipticity for an infinite helix, –40 000 deg cm^2 dmol^{-1} (Chen et al., 1974), was used in this calculation since the Lifson–Roig model accounts for the frayed ends in helical peptides of modest length.

The s values for each of the four residues in the host peptide, A, E, K, and Y, were determined first from the measured

Table III: Comparisons of Residue Helix Parameters[a]

guest residue	peptides of defined sequence 1	2	3	peptides of undefined sequence 4
A	1.81	1.99	2.19	1.07
C	0.43			0.95
D	0.23			0.75
E	0.73			1.47
F	0.79			1.05
G	0.05	0.02	0.57	0.51
H	0.37			0.68
I	0.43	0.44	1.02	1.26
K	1.29			0.86
L	1.03	1.70	1.55	1.10
M	0.79	0.87	1.41	1.18
N	0.23		0.73	0.74
Q	0.58	0.61	1.20	1.01
R	1.94			1.03
S	0.29		0.86	0.70
T	0.18		0.79	0.76
V	0.18	0.20	0.93	0.86
W	0.58			1.13
Y	0.43			1.02

[a] The numbers in columns 1, 2, and 3 are s values obtained from analysis of peptides of defined sequence using the Lifson–Roig algorithm and assuming all residues have a common nucleation parameter, σ, of 0.003. The values listed in column 1 were obtained in this study by extrapolation of dichroic measurements in KCl solutions at pH 7.0 and 0 °C. The values listed in column 2 were obtained from dichroic measurements at pH 7.0 in 1.0 M NaCl by Padmanabhan et al. (1990) and Chakrabartty et al. (1991) and calculated by Chakrabartty and Baldwin (1993). The values listed in column 3 were obtained from dichroic measurements at pH 7.0 in 0.01 M KF by Lyu et al. (1990) and Gans et al. (1991) and calculated by Chakrabartty and Baldwin (1993). The values listed in column 4 are s values obtained from dichroic analysis of peptides of undefined sequence at 0 °C using the Zimm–Bragg algorithm and allowing the nucleation parameter to vary (Wojcik et al., 1990).

helix contents of the four peptides containing these guest residues. Their fitted s values appear to represent global minima, since the same values are obtained irrespective of the starting value for each residue in the fitting procedure. The s values for these four residues were then held constant, and an s value was fit to the guest residue in each of the remaining host/guest peptides. The resulting fitted s values are listed in Table III.

These fitted s values are smaller than the s values previously reported for some neutral guest residues in alternative host peptides as shown in Table III. The mean difference and standard deviation between the fitted s values and those calculated from the measurements of Baldwin and co-workers (excluding L) and of Kallenbach and co-workers are 0.05 (0.07) and 0.57 (0.09), respectively. These relatively constant differences likely reflect the varied treatment of the electrostatic and lyotropic contributions to the dichroic measurements of these host/guest peptides.

As has been previously noted (Scholtz & Baldwin, 1992), residue parameters obtained from dichroic measurement of host/guest peptides of defined sequence are at variance with the analysis of peptides of undefined sequence, as illustrated in Table III. The peptides of undefined sequence were analyzed using the Zimm–Bragg model, allowing both the nucleation and propagation parameters of each residue to vary (Wojcik et al., 1990). Surprisingly, the nucleation parameters encompass a wide range, from 0.00001 to 0.0210, while the propagation parameters have a narrow range, from 0.51 to 1.47, with discordant thermal coefficients. These ranges suggest that nucleation rather than propagation is the dominant factor in helix formation. By contrast, the opposite is considered in the analysis of peptides of defined sequence,

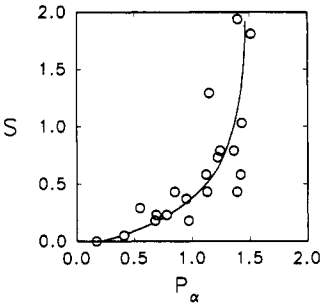

FIGURE 6: Comparison with protein helix frequency measurements. The abscissa is the normalized frequency of residues occurring in the middle of helical structures in protein crystallographic models (Wishart, 1991). A random occurrence has frequency of 1.0 and a more frequent occurrence a value greater than 1.0. The ordinate is the s value fit to the guest residue of each host/guest peptide used in this study supplemented by an s of 0.01 for proline calculated from the ellipticity measurements of Merutka et al. (1990).

Table IV: Prediction of Peptide Helical Content[a]

peptide sequence	% helical content predicted	measured	difference
acetylGELEELLKKLKELLKGamide	9	21	−12
acetylAETAAAKFLRAAAamide	31	26	5
acetylAAQAAAAQAAAAQAAYamide	57	47	10
acetylYKAAAAKAAAAKAAAAKamide	71	67	5

[a] The first peptide has been utilized in studies of the stabilization of four-helix bundles by Ho and DeGrado (1987). The mean residue ellipticity of the monomeric peptide was extrapolated from values measured in 1.5–2.5 M guanidine hydrochloride. The second peptide is an analog of the C-peptide of ribonuclease designed by Fairman et al. (1989) to eliminate the interaction between F8 and H12 in the parent C-peptide. Its mean residue ellipticity was extrapolated from measurements in NaCl. The third peptide was designed to minimize residue interactions by Scholtz et al. (1991). Its mean residue ellipticity was extrapolated from measurements in NaCl, Na_2SO_4, and $CaCl_2$. The last peptide is the host peptide for the host/guest measurements reported by Padmanabhan et al. (1990) and by Chakrabartty et al. (1992). Its mean residue ellipticity was estimated from the value measured in 1.0 NaCl using the mean slope reported in this study. All percent helical content values were obtained assuming the helix and coil forms of each peptide have mean residue ellipticity values of −40 000 and +300 deg cm^2 dmol^{-1}, respectively, at 222 nm, 0 °C, and pH 7.0.

based on the similarity of the range of ϕ,ψ angles available to all residues except for glycine and proline.

The s values obtained from the host/guest peptides of defined sequence used in this study exhibit a general curvilinear correlation with normalized residue helix frequency values obtained from analysis of protein crystallographic models (Wishart, 1991), as illustrated in Figure 6. The complexity of this correlation results in part from an anticipated nonlinear relationship of the two variables and in part from a recognition that protein helix frequency values may also reflect the ability of helical residues to be compactly integrated into the tertiary and quaternary structures of proteins.

The s values obtained in this study were used to predict the helical content of several peptides of defined sequence whose mean residue ellipticities have been measured in solvents of high ionic strength. The predicted and measured helical contents of these peptides are compared in Table IV. The predicted values are in general rather good, suggesting that these s values may have some general currency. However, it should be recognized that these s values were minimally determined and that nonelectrostatic intrapeptide interactions may have perturbed their evaluation. It is anticipated that the correspondence of predicted and measured helix contents will improve as the s values are refined.

Electrostatic Interactions. The electrostatic interactions in the host/guest peptide in this study principally involve

interactions among the ionic side chains and the partial charges on the helical frayed ends. The negative $\Delta\Delta G_{m-e}$ values observed for the host peptides containing neutral guest residues at pH 7.0 and 0 °C (Tables I and II) most likely reflect the contributions of the $i,i+4$ ion-pair interactions among the ionic side chains. To a first approximation, the partial charges on the frayed ends should be complemented continuously by the ionic residues located in each frayed end and by the mobile counterions in solvents of increasing ionic strength. The three potential complementary $i,i+4$ ion-pairs in the peptides having neutral guest residues could then be cumulatively assigned a mean ΔG contribution to helix stability of –0.19 kcal/mol at 0 °C in the absence of added salt. It is likely that the $i,i+4$ ion-pair involving residues 7 and 11 is the primary contributor to helix stability since both partners in this ion-pair are uniquely located in the central portion of the helix.

By contrast, the electrostatic interactions observed at pH 2.0 and 0 °C destabilize the helix in peptides having neutral guest residues by 0.20 kcal/mol, as indicated in Table II. Such destabilization likely results from the unpaired partial positive charge in the N-terminal frayed end of the peptide. This charge only becomes complemented by mobile counterions in solvents of increasing ionic strength.

The presence of an ionic residue in the guest position does not perturb the electrostatic stabilization of the helical conformation of the host/guest peptides at neutral pH significantly, as indicated by the mean $\Delta\Delta G_{m-e}$ values at pH 7.0 listed in Table II. This suggests that, within the precision of these measurements, the $i,i+3$ complementary and antagonistic electrostatic interactions involving the host lysine residue at position 6, an ionic guest residue at position 9, and the host glutamate residue at position 11 are equivalent. However, this is not the case at acidic pH, as indicated by the enhanced mean $\Delta\Delta G_{m-e}$ values observed for basic guest residues at pH 2.0 in Table II. Such enhancement indicates that the $i,i+3$ antagonistic electrostatic interaction between the host lysine residue at position 6 and a guest basic residue at position 9 diminishes the helical content of these host/guest peptides. This diminution is responsible for the enhanced mean $\Delta\Delta G_m$(pH 2–7) value listed for the basic guest residues in Table II.

Effect of Ionization on the s Value. As discussed under Results, the persistent negative $\Delta\Delta G_e$(pH 2–7) observed for all the host/guest peptides (Table II) appears to originate in the s values for the constituent acidic residues. The ΔG_e values for the host/guest peptides measured at pH 2.0 correspond to an s value for a glutamatic acid residue of 0.87. This value is 0.14 larger than the s values measured for that of a glutamate residue which is listed in Table III.

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