Modulation of the Helical Stability of a Model Peptide by Ionic Residues†

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ABSTRACT: The mean residue ellipticity of the helical host peptide, acetyl-YEAAAKEAXAKEAAAKA-amide containing guest residues at position X, was measured as a function of pH and ionic strength at 0 °C. Changes in ellipticity at 222 nm were interpreted in terms of a two-state helix/coil transition of a monomeric peptide. Variable pH measurements in low concentrations of KCl defined changes in helix stability resulting from the ionization of each guest residue. Variable [KCl] measurements at fixed pH generated ellipticity values for the neutral and ionic forms of each guest residue free of electrostatic and lyotropic contributions. These ellipticity values were used to calculate a helix propagation parameter for each form of a guest residue using the Lifson–Roig algorithm and assuming a universal nucleation parameter. In all cases, the propagation parameter of a residue is either unaffected or decreased by ionization of its side chain.

The ionization of an amino acid residue can markedly alter the helix/coil equilibrium of model helical peptides. Such alterations may result from the formation of ion-pair electrostatic interactions either with other ionic residues spaced $i, i + 4$ in the peptide sequence (Marqusee & Baldwin, 1987; Lyu et al., 1989) or with the partial charges in each helical frayed end (Ihara et al., 1982; Shoemaker et al., 1987; Fairman et al., 1989; Takahashi et al., 1989). However, recent measurements indicate that the ionization of an amino acid residue can also alter the helix/coil equilibrium of model peptides in the presence of high ionic strength solvents, which presumably screen out such electrostatic interactions (Park et al., 1993). This observation suggests that ionization of a residue may change its inherent helical propensity parameters, particularly the propagation parameter s.

The sequence of the peptide acetyl-YEAAAKEAAAKE-AAAKA-amide contains several design features which make it useful as a host peptide for the measurement of helix/coil equilibria (Marqusee & Baldwin, 1987; Park et al, 1993). A tyrosine residue has been placed at the N-terminus to provide a unique chromophoric residue for measurement of peptide concentration. The interior residues have been selected so that the peptide is water-soluble and exhibits a significant helical population in a wide range of solvent conditions. Residues having ionic side chains are distributed throughout the sequence, so that the surface of the helical conformation does not contain extensive apolar patches that would facilitate peptide association at any pH. Finally, replacement of the central alanine residue with each of the remaining 19 common residues produces distictive changes in the helix/coil equilibria.

In this report, we examine the effect of the ionization of arginine, aspartate, cysteine, glutamate, histidine, lysine, and tyrosine guest residues on the helix/coil equilibrium of the model host peptide, acetyl-YEAAAKEAXAKEAAAKA-amide, in which X denotes the guest residue position. Each host/guest peptide was titrated in a low ionic strength solvent to measure the apparent pK and the change in mean residue ellipticity associated with each pH transition. The electrostatic contributions to the observed mean residue ellipticity were then screened out at selected pH values at which the neutral or the ionic form of each guest residue predominates. A helix propagation parameter for each form of each guest residue was then fit to the collective fractional helix contents of all of the host/guest peptides measured at discrete pH values using the Lifson–Roig algorithm (Lifson & Roig, 1961; Qian & Schellman, 1992).

EXPERIMENTAL PROCEDURES

Materials. All peptides were synthesized from (*tert*-butyloxycarbonyl)amino acids (tBOC) by the simultaneous multiple peptide synthetic procedure (Houghten et al., 1986), purified by reversed-phase chromatography, and subjected to analytical reversed-phase chromatrography, amino acid compositional analysis, and FAB mass spectrometry, as described previously (Park et al., 1993). 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 deviations 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 of 11.1 mM^{-1} cm^{-1} (Mihalyi, 1968).

Dichroic Measurements. All circular dichroic measurements were made using an Aviv Model 62DS spectrometer equipped with a thermoelectric temperature controller and standardized as described previously (Park et al., 1993). Routine peptide dichroic measurements were made at 222 nm at 0 °C in solutions containing 20–450 μM peptide, 1 mM phosphate buffer, and the indicated concentrations of KCl using rectangular cells having optical paths of 1–10 mm. All ellipticity measurements are reported as mean residue ellipticity, [θ], having the units deg cm^2 dmol $residue^{-1}$. The mean residue ellipticity of representative host/guest peptides

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was found to be independent of peptide concentration at all pH values within the standard deviation of the ellipticity measurements, 500 deg cm^2 dmol^{-1}. This independence suggests that no significant population of polymeric forms of the peptide accumulate in solution.

The peptides used in this study exhibit far-ultraviolet circular dichroic spectra having minima at 222 and 208 nm, which is characteristic of peptide solutions containing a significant concentration of residues in an α-helical conformation. Changes in the pH or the [KCl] of each peptide solution generate a nested series of spectra exhibiting an isodichroic value at 203 nm, consistent with the occurrence of a two-state helix/coil transition. The mean residue ellipticity values at 222 nm, $[\theta]_{obsd}$, can be expressed as ΔG values for helix formation using eq 1. When using eq 1, the mean residue

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

ellipticities of the helix and coil conformations, $[\theta]_{helix}$ and $[\theta]_{coil}$, were assumed to be −35 600 and +300 deg cm^2 dmol^{-1}, respectively (Merutka et al., 1990). The contribution of the helical frayed ends to the calculation of ΔG for the host/guest peptides is incorporated in the mean residue ellipticity value assumed for the helical conformation. The standard deviation for replicate measurements of the ΔG for a host/guest peptide is 0.03 kcal/mol. The mean residue ellipticity values of the helix and coil conformations were increased by 1200 deg cm^2 dmol^{-1} for host peptides containing aromatic guest residues to account for the contributions of these chromophoric side chains (Sears & Beychok, 1973; Brahms & Brahms, 1980). These values were decreased by 800 deg cm^2 dmol^{-1} to account for the contribution of the phenolic form of each tyrosine residue in the host/guest peptides.

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. The pH of the peptide solutions was changed incrementally by additions of solutions of HCl or KOH. All of the pH transitions were observed to be reversible.

Calculation of Propagation Parameters. The propagation parameters for the guest residues were obtained using the Lifson–Roig statistical mechanical model (Lifson & Roig, 1961), in which the nucleation and propagation parameters for each residue are expressed (Qian & Schellman, 1992) in terms of the σ and s values of the Zimm–Bragg model (Zimm & Bragg, 1959), respectively. The mean residue ellipticity measured for each peptide solution was expressed in terms of its mean residue % helicity, assuming limiting mean residue ellipticity values for a coil and an infinite helix of 300 and −40 000 deg cm^2 dmol^{-1} (Chen et al., 1974), respectively. These limiting values were selected since the Lifson–Roig model accounts for the frayed ends in helical peptides of modest length. These limiting values were altered as described above to account for the contributions of aromatic guest residues and the phenolic ionization of tyrosine residues. The nucleation parameter, σ, was held at a constant value of 0.003 for each residue, as is common practice (Scholtz & Baldwin, 1992). The measured % helicity of all peptides was then simultaneously fit with a propagation parameter, s, for each component residue. The program written previously was expanded to simultaneously fit 60 peptides containing each of the 19 host/guest peptides, whose % helicity was measured at either three or four discrete pH values. Fitting was done using the algorithm of Brent (1973) for minimization of χ^2 differences between calculated and observed helical contents.

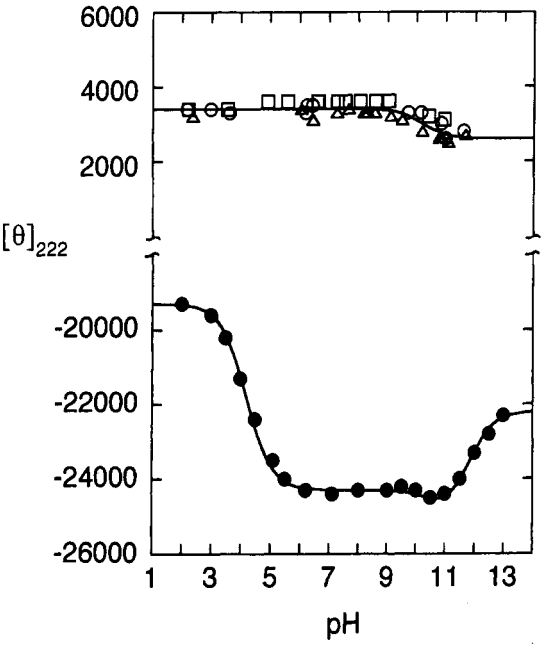

FIGURE 1: Representative dichroic measurements at 222 nm and 0 °C. The filled circles indicate measurements of the host peptide having an alanine guest residue in 0.01 M KCl. The line was drawn assuming three transitions having apparent pK values of 4.2, 10.2 and 11.8 and $\Delta[\theta]$ values of 5000, 800, and 2800 deg cm^2 dmol^{-1}, respectively. The open circles, triangles, and squares indicate measurements of the host peptides having an alanine, a cysteine, and a histidine guest residue, respectively, in 7 M guanidine hydrochloride. The line was drawn assuming a single transition having an apparent pK of 10.1 and a $\Delta[\theta]$ of 800 deg cm^2 dmol^{-1}.

Table I: Assignment of Apparent pK Values[a]

pK value		residue assignment
measured	reference	
4.2 (0.1)	4.2 (0.1)	glutamate
6.9	6.8 (0.3)	histidine
8.6	8.6 (0.2)	cysteine
10.0 (0.2)	10.2 (0.2)	tyrosine
11.9 (0.1)	12.0 (0.2)	lysine
13.5	>12	arginine

[a] The reference pK values for arginine, histidine, and cysteine are for unstructured proteins measured at 25 °C (Cantor & Schimmel, 1980). The remaining values are obtained from ^{13}C NMR analysis of the peptide GGXA at 35 °C (Richarz & Wüthrich, 1978). The value for lysine has been corrected to 0 °C using a thermal coefficient of −0.027 pK deg^{-1} (Bates, 1962).

RESULTS AND DISCUSSION

Effect of pH in 0.01 M KCl. The pH profile of the mean residue ellipticity of the host peptide containing a neutral representative guest residue, alanine, is illustrated in Figure 1. This profile can be fit by three transitions having apparent pK values of 4.2, 10.2, and 11.8. As shown in Table I, these measured pK values are appropriate for transitions involving the glutamate, tyrosine, and lysine residues in the host peptide. Only the transition involving the tyrosine residue persists in the presence of the denaturant, 7 M guanidine hydrochloride, as shown in Figure 1. This persistence reflects a conformationally independent change in ellipticity accompanying the phenolic ionization of a tyrosine residue. The absence of transitions involving the glutamate and lysine residues in 7 M guanidine hydrochloride indicates that these transitions have

Table II: Analysis of Host/Guest Peptide Dichroic pH Profiles[a]

guest residue	acidic transition		other transition		phenolic transition		basic transition	
	pK_{app}	Δ[θ]	pK_{app}	Δ[θ]	pK_{app}	Δ[θ]	pK_{app}	Δ[θ]
neutral								
mean	4.2	−4300			10.0	−900	11.9	−3100
std dev	0.1	500			0.2	200	0.1	700
cationic								
lysine	4.2	−6500			10.2	−2700	11.8	−1600
histidine	4.2	−6300	6.9	−1100	10.2	−800	11.8	−3300
arginine	4.2	−6800	13.5	−300	10.2	−2300	11.9	−1400
anionic								
aspartate	4.6	−2600			10.4	−800	11.7	−3100
glutamate	4.2	−2300			10.2	−800	11.9	−5200
cysteine	4.2	−5400	8.6	5700	10.2	−1000	11.8	−2200
tyrosine	4.2	−4400			10.2	−2700	11.9	−3900

[a] All Δ[θ] values were measured at 0 °C and have the units deg cm^2 dmol^{-1}. A negative Δ[θ] value indicates that helical stability is increased upon ionization of the side chain of the residue considered.

a conformational origin. These conformationally dependent transitions involving the glutamate and lysine host residues will be denoted as the acidic and basic transitions, respectively.

The pH dependence of the mean residue ellipticity has been obtained for host peptides containing 19 different guest residues. The profiles for the host peptides containing neutral guest residues can all be fit by three transitions, whose apparent pK and Δ[θ] values are listed in Table II. The small magnitude of the standard deviations for these values suggests that the pH effects are remarkably uniform.

The pH profiles observed for host peptides containing cationic guest residues are compared with the pH profile observed for the host peptide containing a neutral guest residue in Figure 2A. For purposes of comparison, the [θ] values are presented as Δ[θ] values relative to the [θ] measured for each peptide at pH 13.0, since all peptides having neutral and cationic guest residues should have the same net charge at this pH. The presence of a cationic guest residue increases the Δ[θ] of the acidic transition and decreases the Δ[θ] of the basic transition as shown in Table II. (It should be noted that the histidine host/guest peptide does not contain a cationic guest residue in the pH range of the basic transition.) These changes likely reflect the contributions of the i, $i + 3$ antagonistic electrostatic interaction between a cationic guest residue at position 9 and the cationic host residue at position 6.

The presence of a guest lysine residue does not introduce a transition that can be distinguished from those of the three host lysine residues. However, the presence of a histidine guest residue introduces a unique transition having an apparent pK of 6.9 and a Δ[θ] of −1100 deg cm^2 dmol^{-1}, as shown in Figure 2A and Table II. This apparent pK is appropriate to the imidazole side chain of the guest histidine residue, as shown in Table I. This Δ[θ] has a conformational origin since this transition is not detected in the presence of 7 M guanidine hydrochloride, as shown in Figure 1. The presence of an arginine guest residue also appears to introduce a unique transition having an apparent pK of about 13.5 and a Δ[θ] of −300 deg cm^2 dmol^{-1}, as shown in Table II. The values are approximate at best, owing to the small size of the transition and to its overlap with the basic transition of the three host lysine residues.

As shown in Table II, the presence of a cationic guest residue in the pH range 9–11 (either arginine or lysine) significantly increases the Δ[θ] of the phenolic transition. This increase suggests that the cationic guest residues form an i, $i + 8$ complementary ion-pair interaction with the phenolic form of

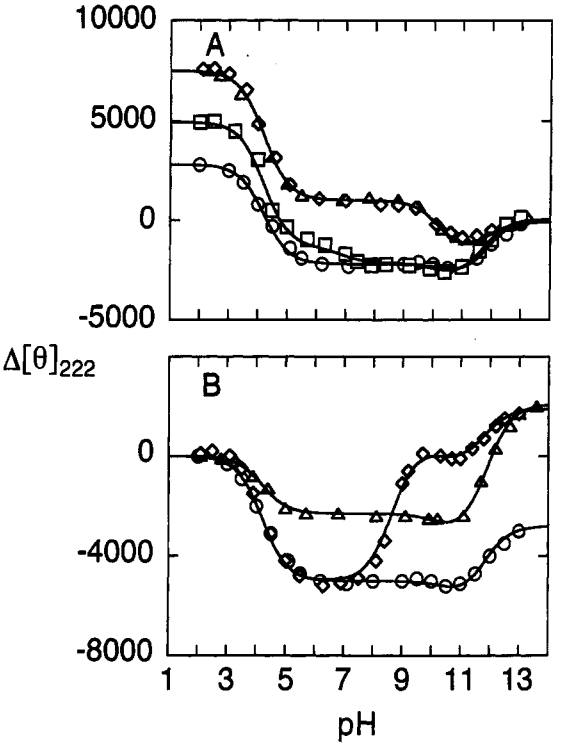

FIGURE 2: Comparative pH profiles for host/guest peptides. All measurements were obtained at 0 °C in aqueous solvents containing 1 mM phosphate and 10 mM KCl. Each line was constructed using the parameters listed in Table II. A compares the pH profiles for host peptides containing representative neutral and cationic guest residues. The symbols indicate the presence of the following guest residues: ○, alanine; □, histidine; △, lysine; ◇, arginine. The Δ[θ] values represent the $[\theta]_{pHobsd} - [\theta]_{pH13}$ measured for each peptide and have the units deg cm^2 dmol^{-1}. B compares the pH profiles of representative neutral and anionic guest residues. The symbols indicate the presence of the following guest residues: ○, alanine; ◇, cysteine; △, glutamate. The Δ[θ] values represent the $[\theta]_{pHobsd} - [\theta]_{pH2}$ measured for each peptide and have the units deg cm^2 dmol^{-1}.

the host tyrosine residue at position 1. Model building indicates that such an interaction is feasible, provided that small nonionic residues such as alanine occupy positions 4 and 5, as is the case in the host peptide. The differences in Δ[θ] values for the phenolic transition exhibited by neutral guest residues and by the lysine and arginine guest residues indicate that the proposed i, $i + 8$ ion pair contributes no more than −0.1 kcal/mol of stability to the helical peptide conformation.

The pH profiles observed for host peptides containing anionic guest residues are compared with the profile observed for the host peptide containing a neutral guest residue in Figure 2B. For purposes of comparison, the [θ] values are represented as Δ[θ] values relative to the [θ] value measured for each peptide at pH 2.0. This reference pH was selected because all peptides containing neutral and anionic guest residues should have the same net charge at pH 2.0. The presence of an anionic guest residue diminishes the Δ[θ] of the acidic transition and produces an irregular change in the Δ[θ] of the basic transition. These changes reflect in part the contribution of the i, $i + 3$ antagonistic ion-pair interactions involving an anionic guest residue at position 9 and the anionic host residue at position 12.

The presence of either an aspartate or a glutamate guest residue does not introduce a transition that can be distinguished from those of the host glutamate residues. However, the presence of a cysteine guest residue introduces a unique transition, as shown in Figure 2B. This transition has an apparent pK of 8.6 and a Δ[θ] of 5700 deg cm^2 dmol^{-1}, as

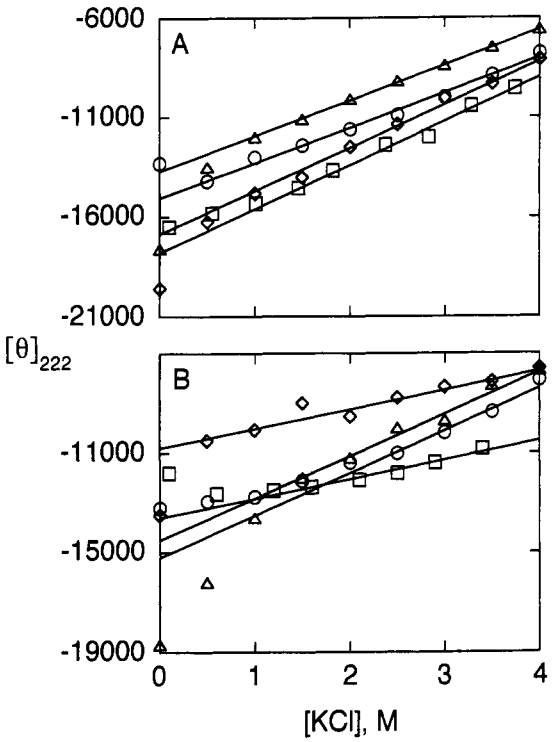

FIGURE 3: Effect of KCl on the mean residue ellipticity of representative host peptides containing ionic guest residues. In both panels, the circles indicate measurements at pH 2, the triangles indicate measurements at pH 7, and the squares indicate measurements at pH 13. A illustrates measurements of the host peptide containing a tyrosine guest residue. The diamonds in this panel indicate measurements at pH 11.0. B illustrates measurements of the host peptide containing a cysteine guest residue. The diamonds in this panel indicate measurements at pH 9.6.

shown in Table II. This apparent pK is appropriate to the sulfhydryl group of the cysteine guest residue as shown in Table I. This unique transition has a conformational origin since it is not detected in the presence of the denaturant, 7 M guanidine hydrochloride, as shown in Figure 1. The presence of a tyrosine guest residue neither introduces a unique transition nor perturbs the acidic or basic transitions, as shown in Table II. However, the $\Delta[\theta]$ of the phenolic transition is more than doubled compared with those of host peptides having neutral guest residues. Such doubling is expected since the phenolic transition is generated by a conformationally independent transition whose $\Delta[\theta]$ is dependent upon the number of tyrosine residues present in the peptide.

Effect of KCl at Constant pH. The electrostatic contributions to mean residue ellipticity values measured in 0.01 M KCl were screened out by the addition of increasing concentrations of KCl to solutions of the host/guest peptides maintained at constant pH. The pH values maintained were selected to represent the beginning and the end of the pH transition of each guest residue and to be as independent as possible of the pH transitions of host residues. Representative results are illustrated in Figure 3.

Each host/guest peptide exhibits a limiting linear relationship in high salt concentrations, which is considered to represent the effect of KCl acting as a lyotropic reagent on the helix/coil equilibria of the host/guest peptides. The slope of the limiting relationship observed for each host/guest peptide was the same at pH 2 and at pH 7. However, the slope of the limiting relationship is somewhat greater at pH 13 for all host/guest peptides, except that containing a cysteine guest residue. This increase in slope correlates with the phenolic ionization of the tyrosine residues, as shown in Figure 3A. By contrast, the slope of the limiting relationship observed for the host/guest peptide containing cysteine is smaller at pH 13 than at pH 7 or 2. This decrease in slope correlates with the ionization of the cysteine guest residue, as shown in Figure 3B.

Table III: Residue Propagation Parameters

	propagation parameter at 0 °C			propagation parameter at 0 °C	
residue	neutral	ionic	residue	neutral	ionic
aspartate	0.24	0.24	histidine	0.21	0.21
arginine	2.30	1.83	lysine	1.45	1.25
cysteine	0.38	0.21	tyrosine	0.45	0.44
glutamate	1.00	0.88			

The difference between the extrapolated and measured mean residue ellipticities in 0.01 M KCl is considered to represent the net contribution of screenable electrostatic interactions to the stabilization of the helical conformation of the host/guest peptides. At pH 2 and 13, the screenable electrostatic interactions destabilize the helical conformation by a mean ΔG of 0.20 and 0.10 kcal/mol, respectively, with a standard deviation of 0.04 kcal/mol. The electrostatic destabilization observed at pH 2 likely results from an unpaired partial positive charge in the N-terminal frayed end of the helix and the destabilization observed at pH 13 from an unpaired partial negative charge in the C-terminal frayed end. The sum of these values, 0.30 kcal/mol, is equivalent to the electrostatic destabilization contributed by the unpaired partial charges in both frayed ends of the peptide acetyl-$(AAQAA)_3$Y-amide (Scholtz et al., 1991).

At pH 7, the partial charge in each frayed end of the host/guest peptides should be complemented by an ionic residue, e.g., glutamate 2 and lysine 16, thus eliminating the electrostatic destabilization observed at pH 2 and 13. The screenable electrostatic interactions at pH 7.0 are observed to stabilize the helical conformation with a mean ΔG of 0.19 kcal/mol and a standard deviation of 0.03 kcal/mol. This stabilization is considered to be contributed principally by the central i, $i + 4$ complementary ion-pair interactions in the host peptide. The stabilization assigned to this ion pair is smaller than previously estimated, 0.4–0.5 kcal/mol (Gans et al., 1991; Stellwagen et al., 1992), but lies within the range of values estimated for the stabilization provided by i, $i + 4$ ion pairs on the surfaces of protein helices (Dao-pin et al., 1991).

Estimation of Propagation Parameters. The mean residue ellipticity obtained by extrapolation of each limiting linear relationship is considered to represent the value for that host/guest peptide free of electrostatic and lyotropic contributions. The extrapolated mean residue ellipticity values for 19 host/guest peptides maintained at either 3 or 4 different pH values, expressed as % helix content, were simultaneously fit with a helix propagation parameter for each residue, using the Lifson–Roig algorithm and the values previously reported (Park et al., 1993) as a starting point. The propagation parameters obtained for the neutral and ionic forms of each residue of interest are listed in Table III. Ionization of each of these residues either decreases its propagation parameter or does not significantly change it. The change in the propagation parameter upon ionization appears related to the magnitude of the propagation parameter of the neutral form, as shown in Figure 4. The slope of this relationship may be artificially large owing to the insensitivity of the mean residue ellipticity of these host/guest peptides to changes in propagation parameters of guest residues greater than 1.0.

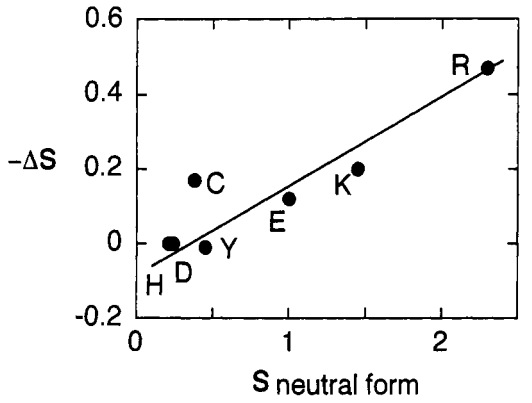

FIGURE 4: Effect of residue ionization on its propagation parameter. Values for individual guest residues, indicated by the one-letter codes, are listed in Table III.

The clear exception to the relationship illustrated in Figure 4 is the host/guest peptide containing a cysteine guest residue. This exception may indicate a particular susceptibility of the hydrogen bonds that stabilizes the central portion of the peptide helix to attack by the uniquely short ionic side chain of this guest residue. The marked change in the lyotropic effect of KCl on this host/guest peptide which accompanies ionization of the cysteine residue, illustrated in Figure 3B, suggests a fundamental change in the structure of this peptide.

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