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Effect of Amino Acid Ion Pairs on Peptide Helicity†

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ABSTRACT: The three ER ion pairs in the peptide acetyl-W(EAAAR)$_3$A-amide were replaced in turn with the ion pairs EK, EO, DR, DK, and DO, where O represents an ornithine residue. The far-ultraviolet circular dichroic spectra of the six peptides measured in 10 mM NaCl at pH 2 and 0 °C form a nested set having an isodichroic point at 203 nm of −17 000 deg cm^2 dmol^{-1}. The ellipticity values of the six peptides at 222 nm range from −31 600 to −7400 deg cm^2 dmol^{-1} in the order listed. Changing the pH of each peptide solution from 2 to 13 also generates a nested set of dichroic spectra with the same isodichroic values. Increasing the pH from 2 to 7 differentially increases the ellipticity at 222 nm in a single transition having an apparent pK of 4.1 for the E-containing peptides are 3.6 for the D-containing peptides. Increasing the pH beyond neutrality differentially decreases the ellipticity at 222 nm in a single transition having an apparent pK of $\geq$13.2 for the R-containing peptides, 11.1 for the K-containing peptides, and 10.7 for the O-containing peptides. It is proposed that the difference in the ellipticity of the six peptides chiefly reflects the helix preferences for the variable residues supplemented by intrahelical electrostatic interactions in the neutral pH range.

Model helical peptides commonly contain acidic and basic residues to increase solubility in aqueous solution and to form favorable interactions with each other and with the helix macrodipole. Marqusee and Baldwin (1987) investigated the effect of the orientation and the spacing of three glutamate/lysine ion pairs on the helical content of a 17-residue monomeric peptide containing 11 alanine residues. They observed that the helical content was optimized at neutral pH when the glutamate/lysine ion pairs were separated by three alanine residues with the glutamate residue being N-terminal. These results can be interpreted to indicate the contribution of salt bridges and helix macrodipole/side-chain electrostatic interactions to helical stability. In this paper, the contribution of different paired acidic and basic residues to helical content is investigated. The peptides studied all have the sequence

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Table I: Mean Residue Ellipticity Values at 222 nm at 0 °C[a]

peptide	$[\theta]$, pH 2	$\Delta[\theta]_a$	pK_a	$[\theta]$, pH 7	$\Delta[\theta]_b$	pK_b	$[\theta]$, pH 13
ER	−32 400	3 000	4.0	−35 400	≥2 000	≥13.2	
EK	−25 700	4 000	4.2	−29 700	2 200	11.1	−27 500
EO	−18 600	12 000	4.2	−30 600	5 800	10.7	−24 800
DR	−15 800	10 500	3.5	−26 300	≥2 500	≥13.3	
DK	−10 800	10 000	3.7	−20 800	5 400	11.1	−15 400
DO	−7 400	17 500	3.7	−24 800	12 000	10.7	−12 800

[a] The subscripts a and b refer to the acidic transitions, respectively.

acetyl-W(XAAAZ)$_3$A-amide in which the acidic residue X and the basic residue Z have the optimal orientation and spacing reported by Marqusee and Baldwin. Residue X is either glutamate, E, or aspartate, D, and residue Z is either arginine, R, lysine, K, or ornithine, O. The six possible combinatorial peptides studied are designed ER, EK, EO, DR, DK, and DO. In a given peptide all three acid residues and all three basic residues are the same; e.g. peptide DK contains three aspartate residues and three lysine residues.

Materials and Methods

Peptide Preparation. All peptides described were synthesized according to the simultaneous multiple peptide synthetic procedure described by Houghten et al. (1986), fractionated by reversed-phase chromatography, and analyzed by FAB mass spectrometry and by analytical reversed-phase chromatography as described previously (Merutka & Stellwagen, 1990). The mass/charge ratio of the main molecular ion of each peptide was within 1 mass unit of that expected for the singly protonated peptide.

Chromatography. Analytical reversed-phase chromatography was done by using a 4.4 × 150 mm Spherisorb ODS2 column and an IBM LC/9533 ternary gradient liquid chromatograph. Samples were partitioned in acetonitrile/water gradients in 0.1% trifluoroacetic acid at ambient temperature at a flow rate of 1 mL/min. One gradient ranged from 10% to 40% acetonitrile developed in 25 min, and a second gradient ranged from 20% to 50% acetonitrile developed in 30 min. The major component in the elution profile of each peptide preparation comprised at least 95% of the eluted material that absorbed at 220 nm. The retention time of the peptides in each gradient is shown in Figure 1.

Spectral Measurements. Circular dichroism and absorbance measurements were made by using Aviv Associates Model 60DS and 14DS spectrometers, respectively, located in the Protein Structure Facility at the University of Iowa. Dichroic measurements were made by using 10-mm Hellma quartz cells placed in a thermostable cell holder. All samples were equilibrated in the spectrometer until the ellipticity at 222 nm was constant prior to the recording of a dichroic spectrum. All ellipticity measurements in this paper are expressed as mean residue ellipticity, $[\theta]$, having the units deg cm^2 dmol^{-1}. Peptide concentrations were calculated from absorbance measurements at 280 nm by using an extinction for a tryptophan residue of 5560 M^{-1} cm^{-1} (Milhalyi, 1968). Mean residue ellipticity values measured for multiple solutions of the same peptide in the same solvent varied by ±3%. All pH and thermal transitions were reversible within the variation of the ellipticity measurements. The mean residue ellipticity at 222 nm of peptide ER in 10 mM NaCl and 1 mM sodium phosphate at pH 7 and 0 °C was independent of peptide concentration over the range 0.43–256 μM.

pH Measurements. All pH titrations were initiated with peptide solutions in 10 mM NaCl and 1 mM phosphate buffer, pH 7.0. The pH of one portion of a peptide solution was lowered by incremental additions of HCl while the pH of another portion of the same peptide solution was raised by incremental additions of NaOH. The pH of these solutions was measured with a Radiometer Model PHM82 pH meter with a Radiometer GK 2421C combined electrode. The instrument was calibrated with standard solutions of pH 2.0, 7.0, and 10.0. The observed pH readings were corrected for sodium ion errors by using information supplied by the manufacturer.

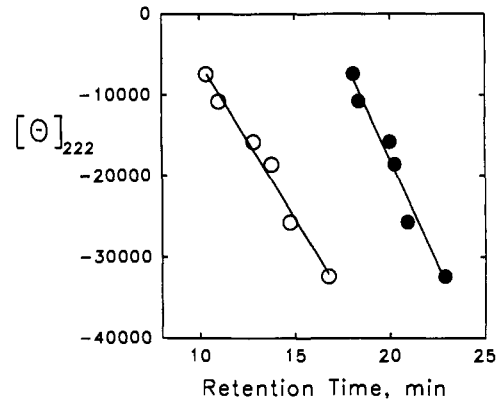

FIGURE 1: Reversed-phase analytical chromatography. The ordinate represents the ellipticity observed for each peptide in 10 mM NaCl at 0 °C and pH 2 as listed in Table I. The abscissa represents the retention time observed for each peptide in each of two acetonitrile/water gradients in 0.1% trifluoroacetic acid at ambient temperature. The open circles indicate a 20–50% acetonitrile gradient developed in 30 min and the filled circles a 10–40% acetonitrile gradient developed in 25 min.

Results

The far-ultraviolet circular dichroic spectrum of each peptide in 10 mM NaCl and 1 mM sodium phosphate at pH 2 and 0 °C forms a nested set as shown in Figure 2A. Such a nested set having an isodichroic point at 203 nm of −17 000 deg cm^2 dmol^{-1} and spectral minima at 222 nm is characteristic for peptides with different fractional helical contents. The ellipticity of each peptide at 222 nm in 10 mM NaCl and 1 mM sodium phosphate at pH 2 and 0 °C is listed in Table I.

The dichroic spectrum of each peptide solution changes as the pH of the solution is increased from 2. The pH-dependent spectral changes for each peptide form a nested set similar to that illustrated for peptide EO in Figure 2B. The dependence of the ellipticity of each peptide solution at 222 nm on the pH is illustrated in Figure 3. The pH dependence of each peptide can be characterized by two unequal transitions, one in the acidic pH range and one in the basic pH range. The acidic transition is assumed to represent the dissociation of E or D residues and the basic transition the dissociation of either the R, K, or O residues. Each transition was fit by using the equation

pH =
apparent pK + log [dissociated form]/[protonated form]

The change in ellipticity and the apparent pK associated with the fitted transitions for each peptide are listed in Table I. The

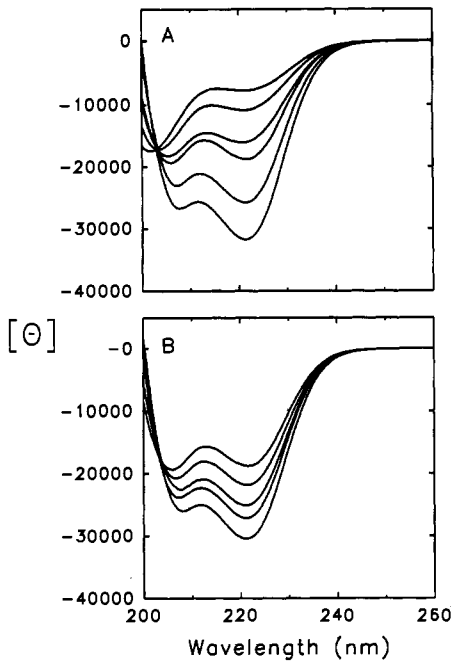

FIGURE 2: Dichroic spectra. Panel A illustrates the dichroic spectra of each of the six peptides observed in 10 mM NaCl at pH 2 and 0 °C. The spectra, reading upward at 222 nm, are for peptides ER, EK, EO, DR, DK, and DO. Panel B illustrates the dichroic spectra of peptide EO in 10 mM NaCl at 0 °C. The spectra were obtained, reading upward at 222 nm, at pH 6.75, 10.78, 11.46, 3.54, and 2.26.

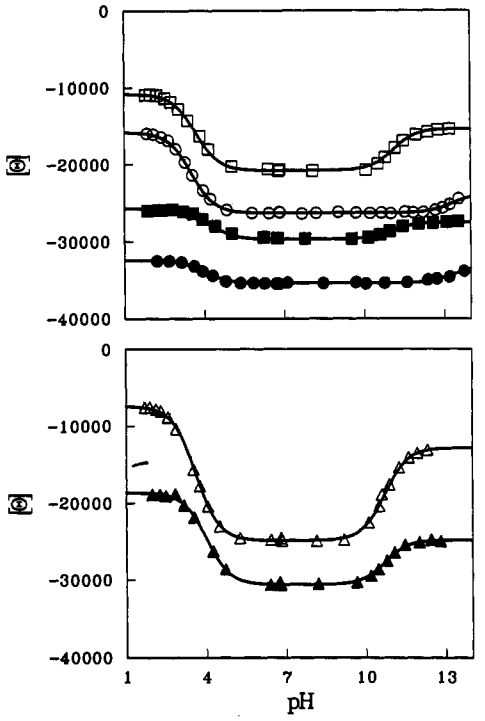

FIGURE 3: Dependence of ellipticity on pH. All measurements were made at 222 nm in 10 mM NaCl at 0 °C. The upper panel illustrates the pH dependence of peptides ER (●), EK (■), DR (○), and DK (□), and the lower panel illustrates the pH dependence of peptides EO (▲) and DO (△). The parameters for the fitted lines are listed in Table I.

parameters for the basic transition in peptides DR and ER can only be estimated owing to the high apparent pK for the R residues.

Increasing the temperature of each peptide solution in 10 mM NaCl and 1 mM sodium phosphate at pH 7.0 alters its dichroic spectrum to form a nested set similar to that illustrated in Figure 2. The spectra obtained for each peptide at

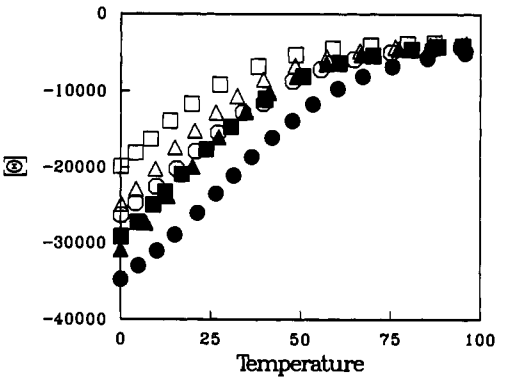

FIGURE 4: Dependence of ellipticity on temperature. All measurements were made at 222 nm in 10 mM NaCl and 1 mM phosphate buffer, pH 7. The dependency is illustrated for peptides ER (●), EK (■), EO (▲), DR (○), DK (□), and DO (△).

Table II: Melting Temperatures in 10 mM NaCl at pH 7.0

peptide	T_m (°C)	peptide	T_m (°C)
ER	35	DR	13
EK	19	DK	-1
EO	23	DO	9

increasing temperature have an isodichroic point at 203 nm with an ellipticity of −17 400 ± 600 deg cm^2 dmol^{-1}. The dependence of the ellipticity of each peptide at 222 nm on temperature is illustrated in Figure 4. The ellipticity of each peptide approaches a common value at elevated temperatures, which likely indicates the dominance of the coil form of each peptide under these conditions. The thermal transition observed for each peptide was fit with a two-state helix/coil model in which the coil form has a thermal coeficient of −33 deg cm^2 dmol^{-1} per °C and the helix form has an ellipticity of −40 500 deg cm^2 dmol^{-1} (Merutka et al., 1990). The fitted thermal transitions form a nested set having a mean ellipticity for the coil form of −500 deg cm^2 dmol^{-1} with a standard deviation of 300, a mean thermal coefficient for the helix of 100 deg cm^2 dmol^{-1} per °C with a standard deviation of 30, and 80% of the transition occurring over 94 °C with a standard deviation of 7 °C. The midpoint or melting temperature for the thermal transition of each peptide is listed in Table II.

Discussion

The spectral comparisons illustrated in Figure 2 indicate that the identity of the acidic and basic residues as well as the pH of the solution markedly influences the helix/coil equilibria exhibited by the six peptides considered in this study. The ellipticity values shown in Figure 3 indicate that the helical content is greatest for peptide ER at neutral pH. We suggest that this principally reflects the superior helix preference of glutamate and arginine residues and the formation of intrahelical electrostatic interactions in the neutral pH range, as described below.

The superior helix preference of glutamate and arginine residues can be demonstrated by comparison of peptide ellipticity values observed at 0 °C or by comparison of melting temperatures obtained by fitting the thermal transitions of the peptides. We have chosen to make these comparisons by pairing the peptides to obtain values for single-residue replacements. For example, a D → E replacement can be estimated in ellipticity units by pairwise comparison of peptides DR and ER, peptides DK and EK, and peptides DO and EO, obtaining the average difference ellipticity for these paired peptides and dividing the average difference ellipticity by 3 to obtain the average difference ellipticity per residue re-

Table III: Effect of Residue Replacements[a]

quantity	pH range	change per replacement		
		D → E	K → R	O → R
$\Delta[\theta]_{222}$	acidic	4700/800	1800/200	3600/1100
	neutral	2800/200	1800/200	3300/600
	basic	4200/200		
ΔT_m (°C)	neutral	6/1	5/1	2/1
$\Delta s'$	acidic	0.64	0.17	
	neutral	0.22	0.17	
ΔP, helical	variable	0.40	−0.27	

[a] The difference ellipticity values, $\Delta[\theta]_{222}$, and the difference melting temperatures, ΔT_m, are presented as the average change per replacement preceding the diagoal line and the standard deviation following the diagonal line. σ values at 0 °C, s', were obtained from Vasquez et al. (1987). Helix probability values, P, were obtained from Levitt (1978). A negative sign indicates that the quantity of the residue being replaced is larger.

placement. The average difference ellipticity values and the average difference melting temperatures for individual replacements in the paired peptides are given in Table III. These values are compared with difference σ values obtained from an extention of the Zimm–Bragg theory to peptides (Vasquez et al., 1987) and with difference helix probability values obtained by analysis of protein crystallographic models (Levitt, 1978). It should be noted that average difference ellipticity values and the average difference melting temperatures correlate with the difference σ values and the difference helix probability values except for the K → R difference helix probability.

The increase in the helical content accompanying the neutralization of each peptide solution (Figure 3) suggests a significant contribution by intrahelical electrostatic interactions for several reasons. First, the change in ellipticity associated with the acidic and basic transitions is quite variable as shown in Table I. This suggests that the change in ellipticity is not due to a change in net charge, which should be constant among all the peptides, but rather is due to considerations that are residue dependent. Second, the σ values for D and E residues (Vasquez et al., 1987) predict that the negative ellipticity of the peptides should decrease rather than increase following neutralization of the acidified peptide solutions. This suggests that an enhanced helical preference for the ionic forms of the acidic and basic residues is not likely responsible for the observed increase in negative ellipticity in the neutral pH range. Third, the apparent pK values for the D and E residues in the peptides (Table I) are significantly less than the pK for model carboxyl side chains such as acetic, propionic, or butyric acid, 4.8 (Dawson et al., 1969), or for model acidic residues such as levulinic acid and monomethylglutaric acid, 4.6 (Tanford, 1962). Similarly, the apparent pK values for K and O residues in the peptides are greater than the pK values for model aminio side chains such as methyl-, ethyl-, or butylamine, 10.6 (Dawson, 1969), or for a model residue 6-aminohexanoic acid methyl ester, 10.4 (Tanford, 1962). These pK differences suggest that the ionic forms of the acidic and basic residues are preferentially involved in intrahelical electrostatic interactions. One interaction consistent with these observations would be formation of intrahelical salt bridges whose strength is dependent upon the steric features of the paired acidic and basic residues. If this be the case, then the larger the change in ellipticity associated with the acidic and basic transition, the stronger the salt bridge. By this criterion, peptide DO, which contains the smallest acidic and basic residues, would generate the strongest salt bridge.

The retention times observed for the six peptides in each of two acidic gradients correlate very well with the mean residue ellipticity of the peptides measured in the acidic pH range, as shown in Figure 1. To a first approximation, the ellipticity scale shown in Figure 1 spans the fractional helical content range from 0.0 to 1.0 for peptide ER at 222 nm (Merutka et al., 1990). The correlation of chromatographic retention time with ellipticity suggests that the helix/coil equilibrium controls the chromatographic partitioning of peptides having the same length, the same net charge, and related sequential features. If this be the case, an alternative simple measure of helical content is available.

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