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Effect of a single aspartate on helix stability at different positions in a neutral alanine-based peptide

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Abstract

A single aspartate residue has been placed at various positions in individual peptides for which the alanine-based reference peptide is electrically neutral, and the helix contents of the peptides have been measured by circular dichroism. The dependence of peptide helix content on aspartate position has been used to determine the helix propensity (*s*-value). Both the charged (Asp^-) and uncharged (Asp^0) forms of the aspartate residue are strong helix breakers and have identical *s*-values of 0.29 at 0 °C. The interaction of Asp^- with the helix dipole affects helix stability at positions throughout the helix, not only near the N-terminus, where the interaction is helix stabilizing, and the C-terminus, where it is destabilizing. Comparison of the helix contents at acidic pH (Asp^0) and at neutral pH (Asp^-) shows that the charge–helix dipole interaction is screened slowly with increasing NaCl concentration, and screening is not complete even at 4.8 M NaCl. Lastly, a helix-stabilizing hydrogen-bond interaction between glutamine and aspartate (spacing i, $i + 4$) has been found. This side-chain interaction is specific for both the orientation and spacing of the glutamine and aspartate residues and is resistant to screening by NaCl.

Keywords: helix dipole; helix propensity; hydrogen bonding

A major objective in the study of charge interactions that affect α-helix stability has been to determine the effects of a single charged residue on α-helix stability when the reference peptide itself is neutral. We report such measurements here for a single aspartate residue inserted at different positions in an alanine-based reference peptide, Ac-$(AAQAA)_3$Y-NH_2. Similar measurements have been made for a single Glu^- residue and for a single Lys^+ residue (Scholtz et al., 1993) and also for a single His^+ residue (Armstrong & Baldwin, in prep.). Studies like this became possible when Scholtz et al. (1991b) found that an electrically neutral alanine-based peptide containing three glutamine residues forms a reasonably stable, monomeric helix in water.

Two effects on helix stability are expected when a charged residue is inserted into a neutral peptide. First, the charged residue interacts with the "helix dipole": the partial charges arising from the point dipoles of the peptide groups. The nature of the macrodipole of the helix has been debated. It may arise from the parallel alignment of the individual peptide dipoles along the helix axis (Wada, 1976) or from the four uncompensated NH groups with partial positive charges at the N-terminus and four CO groups with partial negative charges at the C-terminus (Åqvist et al., 1991). Regardless of the specific molecular description of the macrodipole, the resulting charge distribution can be described by placing the charges of $+0.5q$ close to the N-terminus and $-0.5q$ close to the C-terminus of a helical stretch in a peptide (Wada, 1976; Hol et al., 1978; Sheridan et al., 1982). Here, we use the algorithm of Scholtz et al. (1993), which treats the charge–helix dipole interaction as a simple electrostatic interaction between the charged side chain and two partial charges ($+0.5q$ and $-0.5q$) placed at either end of the helical stretch.

A second effect of a charged side chain on helix stability can occur if the intrinsic helix propensities of the charged (Asp^-) and uncharged (Asp^0) forms are different. Provided only a single charged residue is present in an otherwise neutral helix, these two effects can be separated because they depend differently on the position of the charged residue in the helix. The charge–helix dipole interaction is helix stabilizing when Asp^- is close to the

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N-terminus and it is helix destabilizing close to the C-terminus. The intrinsic helix propensity effect depends on the position of the substituted residue relative to the closest end of the helix, because fraying of the ends of the helix gives rise to the position dependence, and the effect is approximately the same at either end of the helix (Chakrabartty et al., 1991). In investigating these two effects, we use the algorithm developed by Scholtz et al. (1993), which represents both effects within the framework of the Lifson–Roig theory of the helix–coil transition (Lifson & Roig, 1961).

During the course of this work, two other results came into view and were pursued. First, aspartate turned out to be an ideal charged residue for analyzing screening by salt ions of the charge–helix dipole interaction. The reason is that the helix propensities of charged (Asp^-) and uncharged (Asp^0) aspartate are equal within error. Consequently, the magnitude of the charge–helix dipole interaction can be determined simply by comparing the helix contents of a peptide at pH 7 (Asp^-) and pH 2 (Asp^0). This has also been done both at 0.01 M and 4.8 M NaCl. Second, evidence was found for an unexpected helix-stabilizing hydrogen-bond interaction between Gln and Asp spaced (i, $i + 4$). Additional peptides were made to test the proposed interaction, and its properties were compared at pH 2 (Asp^0) and pH 7 (Asp^-).

Results

Peptide design and helix-forming properties

The first 10 sequences in Table 1, the AQ peptides, are based on the design of Scholtz et al. (1991b). The other four sequences, the AQ* peptides, have a slightly modified version of this design where an alanine is removed from a position near the C-terminus and inserted between the first two glutamines. All peptides are 16 residues in length, have three glutamines spaced evenly along the helix, and have a tyrosine at the C-terminus to facilitate accurate concentration determination using the tyrosine extinction coefficient of Brandts and Kaplan (1973). (The measurements described here were completed before Chakrabartty et al. [1993] found an effect of a C- or N-terminal Tyr residue on the CD spectrum of a peptide helix.) The N- and C-termini are capped with an acetyl and amide group, respectively, to prevent unfavorable charge–helix dipole interactions (Shoemaker et al., 1985, 1987; Fairman et al., 1989). For this study, an aspartate is substituted for an alanine at each of several single positions along each sequence (see Table 1). The benefit of this design is that the three glutamines, spaced (i, $i + 5$) or (i, $i + 6$), can solubilize the peptides in H_2O without adding charge to the sequence.

Table 1. *Aspartate at various positions within neutral peptide hosts*

Name	Sequence
D1	Ac-DAQAAAAQAAAAQAAY-NH_2
D2	Ac-ADQAAAAQAAAAQAAY-NH_2
D4	Ac-AAQDAAAQAAAAQAAY-NH_2
D6	Ac-AAQAADAQAAAAQAAY-NH_2
D7	Ac-AAQAAADQAAAAQAAY-NH_2
D9	Ac-AAQAAAAQDAAAQAAY-NH_2
D10	Ac-AAQAAAAQADAAQAAY-NH_2
D12	Ac-AAQAAAAQAAADQAAY-NH_2
D14	Ac-AAQAAAAQAAAAQDAY-NH_2
D15	Ac-AAQAAAAQAAAAQADY-NH_2
D5*	Ac-AAQADAAAQAAAAQAY-NH_2
D6*	Ac-AAQAADAAQAAAAQAY-NH_2
D7*	Ac-AAQAAADAQAAAAQAY-NH_2
D8*	Ac-AAQAAAADQAAAAQAY-NH_2

The helix-forming properties of the AQ reference peptide have been characterized previously by Scholtz et al. (1991b) (for review, see Scholtz & Baldwin, 1992), and the aspartate-substituted peptides show similar helix-forming properties. Thermal unfolding curves are coincident at two peptide concentrations, which differ by 10-fold for each of several peptides tested. The coincidence of the complete transition curves shows that helix formation is not caused by peptide association and probably is monomeric. An earlier study of an alanine-based peptide by sedimentation equilibrium showed that helix formation is monomeric (Padmanabhan et al., 1990).

Dependence of helix content and pK_{obs} on the positions of Asp^0 and Asp^-

The dependence of helix content on the position of aspartate substitution in the AQ peptides at pH 2.0–2.5 is shown in Figure 1A. The data for Asp^0 are fitted to the Lifson–Roig theory, as described in the Materials and methods. The corresponding data shown in Figure 1B include both the effects of the intrinsic helix-forming tendency of Asp^- and of the charge–helix dipole interaction. The solid curve, which represents the best fit of the Lifson–Roig model to the pH 2 data, is included in Figure 1B for comparison. The pH 7 data are fitted to an algorithm describing both the charge–helix dipole interaction and the fraying of the helix ends, according to the Lifson–Roig helix–coil transition theory (Scholtz et al., 1993). The parameters that best fit the data are an s-value of Asp^- of 0.29 (the same value determined for Asp^0) and a dielectric constant between 55 and 75 Debye. The model and the data analysis are described in the Materials and methods. The data points for peptides D7 and D12 in Figure 1A and B are shown on the graph but are excluded from all fits for reasons described below. All other data are fitted satisfactorily by these parameters.

The strength of the charge–helix dipole interaction is given by the difference between the observed pK_a for

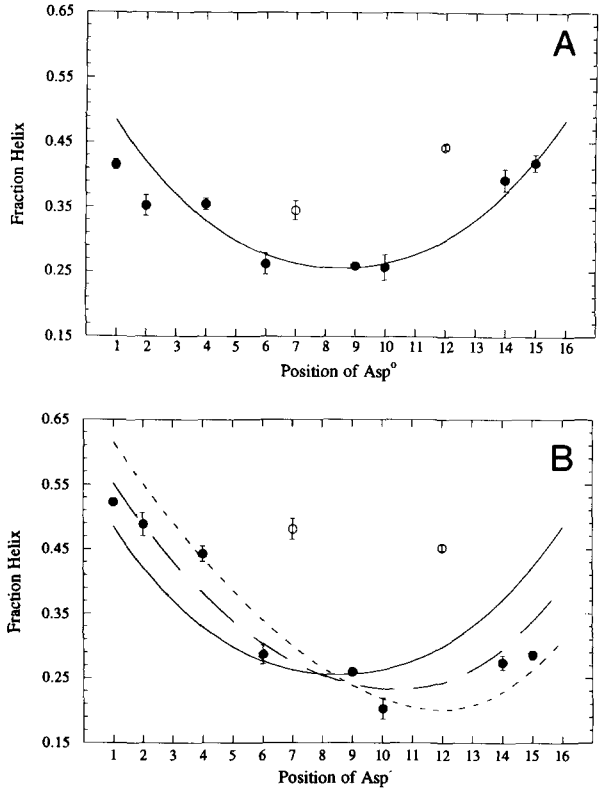

Fig. 1. A: Dependence of helix content on position of Asp^0 at pH 2 in 10 mM NaCl at 0 °C. Peptides used in the fit (●). Peptides excluded from fit (○). **B:** Dependence of helix content on position of Asp^- at pH 7.0 in 10 mM NaCl at 0 °C. The solid line is the fit to the data for Asp^0 in Figure 1A. The dashed lines are the fit to the data using the algorithm of Scholtz et al. (1993) (see Materials and methods) with a dielectric constant of 40 (– – –) and 80 (————) Debye.

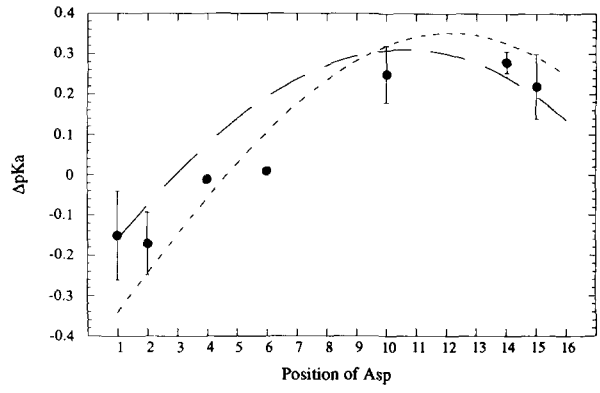

Fig. 2. Dependence of ΔpK_a on the position of Asp using the fitting parameters and experimental conditions of Figure 1. The value for pK_{coil} is 3.91. The dashed lines are the fit to the data using the algorithm of Scholtz et al. (1993) (see Materials and methods) with a dielectric constant of 40 (– – –) and 80 (————) Debye.

Asp in each peptide, pK_{obs}, and the pK_a for Asp in an unstructured peptide, pK_{coil}, where no charge-helix dipole interaction exists. The dependence of ΔpK_a ($pK_{obs} - pK_{coil}$) on the position of Asp in the AQ sequence is shown in Figure 2. A pK_{coil} of 3.91 ± 0.02 was determined by measuring the chemical shift of the β-methylene resonances of Asp by NMR, as described in the Materials and methods, for an unstructured peptide, Ac-AADAA-NH_2, and then fitting the data to the Henderson-Hasselbalch equation. The pK_{obs} value for each helical peptide was determined by measuring the dependence of $-[\theta]_{222}$ on pH and fitted using the Henderson-Hasselbalch equation. We were unable to determine the pK_{obs} values for peptides D9 and D12 because they gave no change in $[\theta]_{222}$ with pH. The algorithm of Scholtz et al. (1993) has also been used to model the dependence of ΔpK_a on the position of Asp (see the dash lines in Fig. 2) using the same parameters found from modeling the dependence of helix content on position of Asp^- (Fig. 1B) (see Materials and methods).

The fraction helix and pK_{obs} values for each peptide in Figures 1 and 2 are averages of at least three different determinations using different stocks of peptide, and the error bars represent the standard deviation from the mean. An exception is the pK_{obs} value for D6, which was measured only once because it has a relatively small change in ellipticity with pH (1,000 deg cm^2 $dmol^{-1}$). All the pK_{obs} values have a larger associated error than the fraction helix values. The pK_{obs} values depend on the magnitude of the change in helicity and on the number of data points in a pH titration, while fraction helix depends directly on $[\theta]_{222}$.

Dependence of the charge-helix dipole interaction on salt concentration

The helix contents of the AQ and AQ* peptides at NaCl concentrations of 0.01 and 4.8 M at pH 7.0 and pH 2.5 are shown in Table 2. The helix contents at 0.01 M NaCl for the AQ peptides have already been presented in the previous section (Fig. 1A,B). The extent of the charge-helix dipole screening by NaCl is determined by comparing the helix contents at acidic and neutral pH for the AQ peptides. For peptides with Asp at the N-terminus (D2 and D4), the helix content is greater at neutral pH than at acidic pH. The opposite effect is seen when Asp is near the C-terminus (peptides D14 and D15). This observation in Table 2 is demonstrated graphically in Figure 3, where the difference in ellipticity between pH 7 and pH 2 is shown as a function of the position of Asp substitution. The line for $\Delta[\theta]_{222} = 0$ is drawn only to aid viewing.

Helix-stabilizing hydrogen-bond interactions in D7 and D12

Peptides D7 and D12 show higher helix contents, both at pH 7.0 and 2.5, than expected from the helix propensities of Asp^0 and Asp^- and the charge-helix dipole inter-

Table 2. *Dependence of the helix-stabilizing effect of the charge–helix dipole interaction on NaCl concentration*

	$-[\theta]_{222}{}^{a} \times 10^{-3}$			
	0.01 M NaCl		4.8 M NaCl	
Peptide	pH 7.0	pH 2.0–2.5	pH 7.0	pH 2.0–2.5
D1	17.4	13.7	10.2	9.9
D2	16.2	11.6	9.4	8.6
D4	14.6	11.6	8.0	7.4
D6	9.2	8.4	5.9	5.8
D7	15.9	11.2	7.5	6.0
D9	8.3	8.3	5.9	6.0
D10	6.1	7.8	5.6	6.1
D12	14.9	14.6	8.0	7.6
D14	8.8	12.9	8.5	9.5
D15	9.2	13.8	9.1	10.0
D5*	13.5	11.5	7.7	7.6
D6*	9.2	9.3	5.8	6.6
D7*	16.6	11.6	8.0	6.6
D8*	7.4	7.4	5.0	5.1

[a] Mean residue ellipticity (deg cm^2 dmol^{-1}) at 0 °C.

action (Fig. 1A,B). Both peptides D7 and D12 have Gln and Asp spaced (i, $i+4$) in the peptide sequence (Table 1). This observation suggests that a hydrogen-bond interaction occurs between these two residues. There is also an (i, $i-1$) spacing between Gln and Asp in the D7 and D12 peptides, and we must consider the possibility that the increased helix stability is caused by an interaction with the (i, $i-1$) spacing. Therefore, we designed a different reference sequence (AQ*), where a single peptide has either the (i, $i-1$) or (i, $i+4$) spacing, but not both (Table 1). The helix contents of D7, D12, and the AQ* peptides are shown in Table 2. The results show that the helix enhancement results from an interaction between Gln and Asp in the (i, $i+4$) spacing. Peptide D7*, which has a spacing of (i, $i+4$) between Gln and Asp, has the expected higher helix content. On the other hand, D8*, which has an (i, $i-1$) spacing between Gln and Asp, does not have a higher helix content. Peptides D5* and D6* were also studied to give the behavior of peptides lacking the interaction but having the same reference sequence.

The effect of the Gln–Asp hydrogen-bond interaction can also be seen in Figure 4, which shows the dependence of helix content on NaCl for two peptides, one that lacks the hydrogen-bond interaction (D6, Fig. 4A) and one that contains this interaction (D7, Fig. 4B). Because the Asp substitution in peptides D6 and D7 is close to the center of the helix, the contribution to helix stability from the charge–helix dipole interaction is small in these peptides. Moreover, because the s-values for Asp^- and Asp^0 are the same, this effect does not contribute to the differences between helix contents at pH 7.0 and 2.5. Therefore, the NaCl screening curves at pH 7.0 and 2.5 should converge at relatively low concentrations of NaCl. This is the case for peptide D6 (Fig. 4A) but not for peptide D7. The pH 7.0 and 2.5 curves for D7 do not converge even at 4.8 M NaCl (Fig. 4B). This result indicates that the Gln–Asp hydrogen-bond interaction is present in peptide D7, that it has different values at pH 7.0 and 2.5, and that it is resistant to screening by NaCl. In the case of peptide D12, there is a sizable contribution to the helix content from the charge–helix dipole interaction, and the helix

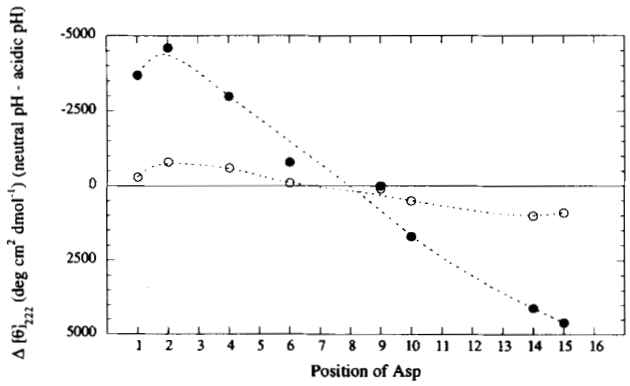

Fig. 3. The difference in $[\theta]_{222}$ between neutral and acidic pH for each peptide at 0.01 M (●) and 4.8 M (○) NaCl at 0 °C. The lines through the data points are drawn by inspection.

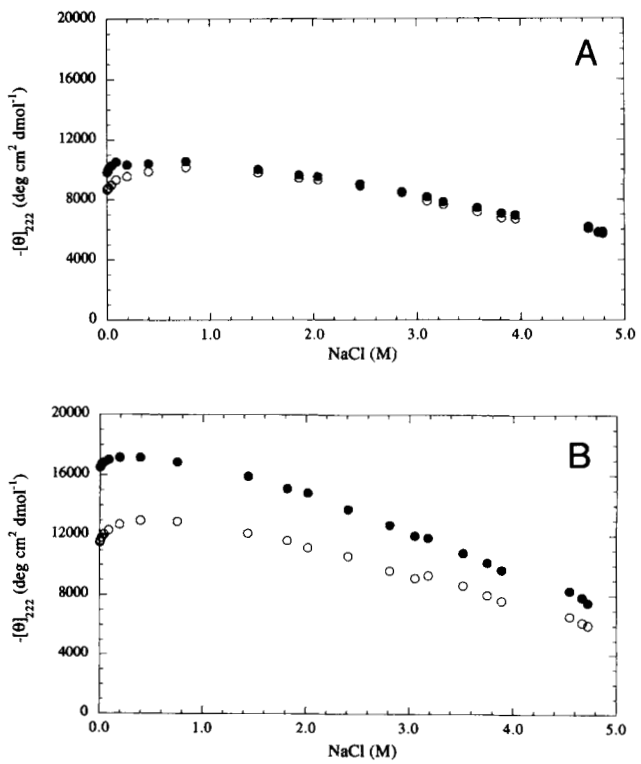

Fig. 4. Dependence of $-[\theta]_{222}$ on NaCl concentration for peptides D6 (**A**) and D7 (**B**) at 0 °C (see Results). At pH 2.5 (○); at pH 7.0 (●).

contents at pH 7.0 and 2.5 are similar both at low and high NaCl concentration (Table 2).

Discussion

Substitution of a single charged residue into a neutral peptide to analyze the helix propensities of Asp^- and Asp^0

In previous studies of the interactions made by charged residues that affect α-helix stability, it was not possible to separate unambiguously the helix-stabilizing effects of ion-pair from charge–helix dipole interactions. The reason was that more than one charged residue was present in all cases, and this resulted in multiple electrostatic interactions that could not be separated straightforwardly from each other. In some studies, multiple charged residues were used to make alanine-based peptides water soluble (Marqusee & Baldwin, 1987, 1990; Marqusee et al., 1989; Huyghues-Despointes et al., 1993). In other studies, multiple ion-pair interactions were used to stabilize a helix (Gans et al., 1991). By using the neutral host peptide introduced by Scholtz et al. (1991b), we are able to distinguish the intrinsic helix-forming properties of a single charged residue from the interactions of this residue with the helix dipole.

The helix propensity of Asp^0 is determined from the dependence of the helix content on the position of the Asp substitution at acidic pH (Fig. 1A). This method was described by Chakrabartty et al. (1991); they used the Lifson–Roig theory to analyze the helix–coil transition and to measure the helix propensity of glycine. The effect of substituting a helix-breaking residue depends strongly on its position in the helix. The effect is larger at the center of the helix than at either end because the ends of short helices fray in water. The resulting value of the helix propensity for Asp^0 ($s = 0.29$, 0 °C) is the smallest of any ionizing residue studied thus far by substitution into a neutral peptide: Glu^0 ($s = 0.62$, 0 °C, Scholtz et al., 1993) and His^0 ($s = 0.36$, 0 °C, Armstrong & Baldwin, in prep.) have higher values of s.

A useful result of these experiments is that the helix propensity of Asp^- can be assessed by comparing the two curves showing helix content versus position of Asp^0 or Asp^- (Fig. 1A,B). The curves cross near the center (position 8.5) of the 16-residue peptide. This indicates that the helix propensity of Asp^- is quite close to that of Asp^0. The pH titration of the helix content of peptide D9 shows a very small change as the aspartate residue is titrated, as expected. The peptide D8 was not made because position 8 is occupied by glutamine. If Asp^0 and Asp^- had different helix propensities, one curve would be shifted to higher helix contents relative to the other curve, and the curves would then intersect near either end of the sequence. This is the case for Glu (Scholtz et al., 1993) and for His (Armstrong & Baldwin, in prep.), which have different helix propensities for their charged and uncharged forms.

The reason for the low s-value of Asp^- and Asp^0 remains to be determined, but it is likely that the proximity of the side-chain carboxylate group to the helix backbone is a major factor, allowing a side-chain–main-chain hydrogen bond to be formed. The helix propensity values for Asp^- and Asp^0 are different from the values determined by the host–guest method, which incorporated aspartate, the guest, in a random sequence copolymer with hydroxybutyl-L-glutamine, the host. In a host–guest study of the helix propensities of Asp^- and Asp^0, the s-value for Asp^0 ($s = 0.82$) was found to be higher than that of Asp^- ($s = 0.71$) at 2 °C in water (Kobayashi et al., 1977).

Properties of the charge–helix dipole interaction

The effect of the charge–helix dipole interaction on helix stability can be obtained from the difference between observed helix content and the value expected from the helix propensity of Asp^- and the position of the substituted Asp (Fig. 1B). An algorithm, based on the Lifson–Roig theory of the helix–coil transition and a simple electrostatic model of the charge–helix dipole interaction (Scholtz et al., 1993), is useful in separating s-value effects and charge–helix dipole interactions. The charge–helix dipole interaction is strongest at the ends of the helix. When the Asp^- residue is in the center of the peptide, the charge interacts equally with the oppositely charged ends of the dipole, resulting in a small charge–helix dipole interaction. These results are consistent with the observed dependence of ΔpK_a on position of Asp^- in Figure 2. The pK_{obs} values diverge from the value of pK_{coil} when Asp^- is near the ends of the sequence, whereas they are very close for peptides that have Asp^- at the center of the sequence.

Because the interaction occurs only within the helix, the strength of the charge–helix dipole interaction depends on the extent of helix formation; thus pK_{obs} depends on the fraction helix. When the helix is denatured by increasing temperature, the pK_{obs} values at different positions of the Asp residue approach the value of pK_{coil} (data not shown). Because these peptides are still only partly helical at 0 °C, we see a ΔpK_a of only ±0.2–0.3 at the ends of the helices. Lockhart and Kim (1993) have found ΔpK_a values twice as large for longer and more helical peptides.

It has often been suggested that the charge–helix dipole model arises either from electrostatic interactions or hydrogen-bond interactions. Our data indicate that the charge–helix dipole interaction studied here is electrostatic because a purely electrostatic model can fit the data in Figures 1 and 2. Furthermore, the charge–helix dipole interaction is screened by increasing concentrations of NaCl (Fig. 3), which is a property of electrostatic interactions

but not hydrogen-bond interactions. Previous studies of isolated helical peptides (Ihara et al., 1982; Shoemaker et al., 1987; Fairman et al., 1989; Takahashi et al., 1989) and of proteins (Nicholson et al., 1988, 1991) also indicate that the charge–helix dipole interaction is electrostatic. Crystallographic data of Nicholson et al. (1988, 1991) demonstrate that an aspartate side chain introduced by mutation close to the N-terminus of a helix in T4 lysozyme is not hydrogen bonded, although the mutation results in increased thermal stability of the protein.

Screening of the charge–helix dipole interaction by NaCl

The aspartate system is well suited to analyzing the screening by NaCl of the charge–helix dipole interaction. Because Asp^- and Asp^0 have the same values of s, the change in helix content caused by titrating Asp^- from neutral to acidic pH (giving Asp^0) is caused solely by eliminating the charge–helix dipole interaction. By comparing the difference in helix content between neutral and acidic pH for peptides with Asp^- near the one end of the helix, it can be observed that screening of the charge–helix dipole interaction is not complete even at 4.8 M NaCl (Fig. 3; Table 2).

In Figure 3, the difference in ellipticity between neutral and acidic pH is plotted versus position of the aspartate residue, both at low and high NaCl concentration. The shape of the curve is complex because it includes the effect of fraying at the helix ends as well as the charge–helix dipole interaction. The curve at 4.8 M NaCl, although smaller in magnitude, reproduces the shape of the curve at 0.01 M NaCl and indicates that there is still some residual charge–helix dipole interaction at 4.8 M NaCl. This conclusion is consistent with the earlier observations by Ihara et al. (1982) and Takahashi et al. (1989) of the salt dependence of helix content in double-block copolymers of alanine and glutamate or lysine (20 residues in each block), and of Lockhart and Kim (1993), who demonstrated that ΔpK_a, the difference in pK_a in the presence and absence of helical structure for helical peptides with N-terminal titratable groups, is not completely screened by 4 M NaCl.

A helix-stabilizing hydrogen-bond interaction between Gln and Asp spaced (i, i + 4)

The helicity of peptides D7 and D12 is higher than that expected from effects of helix propensity and charge–helix dipole interactions (Fig. 1). The following evidence indicates that the effect is caused by a hydrogen-bond interaction between Gln and Asp spaced (i, $i + 4$) in the sequence. The same effect is found in three different peptides (D7, D7*, and D12) and in two different peptide systems (AQ and AQ* peptides) and is, therefore, not a peculiar characteristic of a single peptide substitution or reference peptide system. The singly charged hydrogen-bond interaction at pH 7.0 stabilizes the helix more than the uncharged interaction at pH 2.5. The interaction seems to be specific for this orientation of Gln and Asp residues because the possible interaction in D4, D5*, and D9 peptides, which have the reverse orientation and an (i, $i - 4$) spacing between Gln and Asp, is weaker or nonexistent. Similarly, we do not observe any increase in helicity from hydrogen-bond interactions between Gln and Asp spaced (i, $i \pm 3$). Surprisingly, these results are different from data for ion-pair interactions, which show helix stabilization with both orientations and with both (i, $i + 4$) and (i, $i + 3$) spacings (Marqusee & Baldwin, 1987; Huyghues-Despointes et al., 1993; Scholtz et al., 1993). Comparison of the dependences of helix content on NaCl concentration for D7, D7*, and D12 shows that the hydrogen-bond interaction is resistant to screening by salt, whereas ion-pair interactions are readily screened by salt (Scholtz et al., 1993) and charge–helix dipole interactions exhibit intermediate screening by salt (see Fig. 4; Table 2).

The results shown here for an (i, $i + 4$) hydrogen-bond interaction between Gln and Asp provide the first clear evidence that the helix can be stabilized by uncharged or singly charged side-chain hydrogen-bond interactions. Marqusee and Baldwin (1987) suggested that a singly charged hydrogen bond between uncharged Glu and charged Lys was present at low pH, but they were unable to separate this effect from two other effects: (1) the interaction of charged lysine with the helix dipole, and (2) the change in helix propensity of Glu^- when it is protonated to give Glu^0. A similar interaction between Gln and Glu spaced (i, $i + 4$) has been found by Scholtz et al. (1993). Future experiments must be performed to understand the specific orientation and spacing requirements of this side-chain hydrogen-bond interaction.

Materials and methods

Peptide synthesis, purification, and storage

Peptides were synthesized, cleaved, and purified as described by Chakrabartty et al. (1991). Peptide purity was assessed by fast-performance liquid chromatography (FPLC); purity was greater than 95% for each peptide, and peptide identity was confirmed by fast atom bombardment mass spectrometry. The dry peptides were stored in a desiccator at −20 °C. The aqueous stocks were prepared by dissolving the dry peptide in water, adjusting the pH to 7 (if necessary to dissolve the peptide), and centrifuging the solution. These stocks were always used on the same day of the experiment and discarded afterward because the peptides tended to aggregate if stored in water for long periods of time (2–3 weeks). The aggregation phenomenon was detected by taking absorbance readings at 300, 275, and 250 nm. The stock solution was used if the ratios of A_{300}/A_{275} and A_{250}/A_{275} did not exceed 0.02 and 0.20, respectively.

Circular dichroism measurements

An AVIV 60 DS spectropolarimeter equipped with a Hewlett-Packard model 89100A temperature controller and cuvettes with 1-mm or 1-cm pathlengths were used for all CD measurements. The ellipticity is reported as mean molar residue ellipticity, $[\theta]$ (deg cm^2 dmol^{-1}), and was calibrated with (+)-10-camphorsulfonic acid. The degree of helical structure in each peptide was determined by monitoring the ellipticity at 222 nm at 0 °C. Thermal denaturation curves were performed by measuring the ellipticity at 222 nm at 2 °C increments from 0 to 50 °C. Samples for pH titrations and for single pH measurements were prepared by diluting aqueous stock solutions into 10 mM NaCl buffered with 1 mM sodium citrate, 1 mM sodium phosphate, and 1 mM sodium borate (CD buffer). Samples for single pH measurements were prepared in a CD buffer that had the desired pH. The measurements for the pH titrations and the NaCl dependences were performed as described by Huyghues-Despointes et al. (1993). Concentrations of the peptide stock solutions were determined by measuring Tyr absorbance in either water (ϵ_{275} = 1,390 M^{-1} cm^{-1}) or in 6 M guanidine hydrochloride with 20 mM phosphate buffer (ϵ_{275} = 1,450 M^{-1} cm^{-1}) (Brandts & Kaplan, 1973). Both methods gave equivalent values for the concentration within error.

NMR experiments

Samples for the NMR measurements were prepared by making a 1-2-mg/mL peptide solution containing 10 mM NaCl, 7% D_2O, 1 mM TSP (3-(trimethylsilyl)-propionic acid), and 3 mM sodium phosphate. The pH of each of six aliquots of this solution was adjusted to the desired pH, ranging from pH 2 to 7, by adding varying amounts of concentrated HCl. After recording the NMR spectrum, the pH was measured in the NMR tubes at 2 °C, as described for the CD samples (Huyghues-Despointes et al., 1993). For each sample, a ^{1}H-NMR spectrum was acquired on a 500-MHz General Electric GN-Omega spectrometer, using a 6,000-Hz spectral width for data collection. The FID (free induction decay) was the sum of 64 transients collected in 4,096 complex points. Spectra were processed on a Silicon Graphics Personal Iris computer using FELIX (Hare Research, Inc.). The chemical shift of the β-methylene resonances of Asp was measured using TSP as a standard, and taking account of the pH dependence of the chemical shift of TSP (De Marco, 1977). The reported pK_a of Asp in Ac-AADAA-NH_2 is an average of the pK_a values of the eight β-methylene resonances determined by fitting the change in chemical shift with pH to the Henderson-Hasselbalch equation.

Modeling by the Lifson-Roig theory including some modifications

The fraction helix values were calculated from the observed ellipticities by the method of Scholtz et al. (1991a). To analyze the data in Figure 1A, we used the single helical sequence approximation of the Lifson-Roig theory (Lifson & Roig, 1961; Qian & Schellman, 1992). The Lifson-Roig parameters can be converted to the more familiar Zimm-Bragg (Zimm & Bragg, 1959) parameters as described by Qian and Schellman (1992), and, except for this section, the Zimm-Bragg parameters will be used. The conversion formulas are $s = w/(1 + v)$ and $\sigma = v^2/(1 + v)^4$. The data for Asp^0 are fitted using an average w-value for the host peptide, $\langle w_{host} \rangle$, of 1.43 ($\langle s_{host} \rangle$ = 1.35), v^2 of 0.0038 ($\sigma = 0.0030$), and a w-value of Asp^0 of 0.31 ($s = 0.29$). The $\langle w_{host} \rangle$ value represents the average w-value for the AQ host peptide when it is treated as a homopolymer. The v^2 value has been determined previously by Scholtz et al. (1991a) and Rohl et al. (1992) for alanine-based peptides of varying chain lengths in water. A thorough description of the application of the helix–coil model can be found in these references and in Scholtz et al. (1993).

The fraction helix and ΔpK_a data in Figures 1B and 2 were fitted by using a modified version of the Lifson-Roig model, which includes a simple model for analyzing the charge–helix dipole interaction. A description of the model is given by Scholtz et al. (1993).

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References

Åqvist, J., Luecke, H., Quiocho, F.A., & Warshel, A. (1991). Dipoles localized at helix termini of proteins stabilize charges. *Proc. Natl. Acad. Sci. USA 88*, 2026–2030.

Brandts, J.F. & Kaplan, L.J. (1973). Derivative spectroscopy applied to tyrosyl chromophores. Studies on ribonuclease, lima bean inhibitor, insulin, and pancreatic trypsin inhibitor. *Biochemistry 12*, 2011–2024.

Chakrabartty, A., Kortemme, T., Padmanabhan, S., & Baldwin, R.L. (1993). Aromatic side-chain contribution to far-ultraviolet circular dichroism of helical peptides and its effect on measurement of helix propensities. *Biochemistry 32*, 5560–5565.

Chakrabartty, A., Schellman, J.A., & Baldwin, R.L. (1991). Large difference in the helix propensities of alanine and glycine. *Nature 351*, 586–588.

De Marco, A. (1977). pH dependence of internal references. *J. Magn. Reson. 26*, 527–528.

Fairman, R., Shoemaker, K.R., York, E.J., Stewart, J.M., & Baldwin, R.L. (1989). Further studies of the helix dipole model: Effects of a free α-NH_3^+ or α-COO^- group on helix stability. *Proteins Struct. Funct. Genet. 5*, 1–7.

Gans, P.J., Lyu, P.C., Manning, M.C., Woody, R.W., & Kallenbach, N.R. (1991). The helix–coil transition in heterogeneous peptides with specific side-chain interactions: Theory and comparison with CD spectral data. *Biopolymers 31*, 1605–1614.

Hol, W.G.J., van Duijnen, P.T., & Berendsen, H.J.C. (1978). The α-helix dipole and the properties of proteins. *Nature 273*, 443–446.

Huyghues-Despointes, B.M.P., Scholtz, J.M., & Baldwin, R.L. (1993). Helical peptides with three pairs of Asp-Arg and Glu-Arg residues in different orientations and spacings. *Protein Sci. 2*, 80–85.

Ihara, S., Ooi, T., & Takahashi, S. (1982). Effects of salts on the non-equivalent stability of the α-helices of isomeric block copolypeptides. *Biopolymers 21*, 131–145.

Kobayashi, Y., Cardinaux, F., Zweifel, B.O., & Scheraga, H.A. (1977). Helix–coil stability constants for the naturally occurring amino acids in water. 16. Aspartic acid parameters from random poly(hydroxybutylglutamine-co-L-aspartic acid). *Macromolecules 10*, 1271–1283.

Lifson, S. & Roig, A. (1961). On the theory of helix–coil transitions in biopolymers. *J. Chem. Phys. 34*, 1963–1974.

Lockhart, D.J. & Kim, P.S. (1993). Electrostatic screening of charge and dipole interactions with the helix backbone. *Science 260*, 198–202.

Marqusee, S. & Baldwin, R.L. (1987). Helix stabilization by $Glu^{-}\cdots Lys^{+}$ salt bridges in short peptides of de novo design. *Proc. Natl. Acad. Sci. USA 84*, 8898–8902.

Marqusee, S. & Baldwin, R.L. (1990). α-Helix formation by short peptides in water. In *Protein Folding* (Gierasch, L.M. & King, J., Eds.), pp. 85–94. American Association for the Advancement of Science, Washington, D.C.

Marqusee, S., Robbins, V.H., & Baldwin, R.L. (1989). Unusually stable helix formation in short alanine-based peptides. *Proc. Natl. Acad. Sci. USA 86*, 5286–5290.

Nicholson, H., Anderson, D.E., Dao-pin, S., & Matthews, B.W. (1991). Analysis of the interaction between charged side chains and the α-helix dipole using designed thermostable mutants of phage T4 lysozyme. *Biochemistry 30*, 9816–9819.

Nicholson, H., Becktel, W.J., & Matthews, B.W. (1988). Enhanced protein thermostability from designed mutations that interact with α-helix dipoles. *Nature 336*, 651–656.

Padmanabhan, S., Marqusee, S., Ridgeway, T., Laue, T.M., & Baldwin, R.L. (1990). Relative helix-forming tendencies of nonpolar amino acids. *Nature 344*, 268–270.

Qian, H. & Schellman, J.A. (1992). Helix-coil theories: A comparative study for finite length polypeptides. *J. Phys. Chem. 96*, 3987–3994.

Rohl, C.A., Scholtz, J.M., York, E.J., Stewart, J.M., & Baldwin, R.L. (1992). Kinetics of amide proton exchange in helical peptides of varying chain lengths. Interpretation by the Lifson–Roig equation. *Biochemistry 31*, 1263–1269.

Scholtz, J.M. & Baldwin, R.L. (1992). The mechanism of α-helix formation in peptides. *Annu. Rev. Biophys. Biomol. Struct. 21*, 95–118.

Scholtz, J.M., Qian, H., Robbins, V.H., & Baldwin, R.L. (1993). The energetics of ion-pair and hydrogen-bonding interactions in a helical peptide. *Biochemistry*, in press.

Scholtz, J.M., Qian, H., York, E.J., Stewart, J.M., & Baldwin, R.L. (1991a). Parameters of helix–coil transitions theory for alanine-based peptides of varying chain lengths in water. *Biopolymers 31*, 1463–1470.

Scholtz, J.M., York, E.J., Stewart, J.M., & Baldwin, R.L. (1991b). A neutral, water-soluble α-helical peptide: The effect of ionic strength on the helix–coil equilibrium. *J. Am. Chem. Soc. 113*, 1591–1594.

Sheridan, R.P., Levy, R.M., & Salemme, F.R. (1982). α-Helix dipole model and electrostatic stabilization of 4-α-helical proteins. *Proc. Natl. Acad. Sci. USA 79*, 4545–4549.

Shoemaker, K.R., Kim, P.S., Brems, D.N., Marqusee, S., York, E.J., Chaiken, I.M., Stewart, J.M., & Baldwin, R.L. (1985). Nature of the charged-group effect on the stability of the C-peptide helix. *Proc. Natl. Acad. Sci. USA 82*, 2349–2353.

Shoemaker, K.R., Kim, P.S., York, E.J., Stewart, J.M., & Baldwin, R.L. (1987). Tests of the helix dipole model for stabilization of α-helices. *Nature 326*, 563–567.

Takahashi, S., Kim, E., Hibino, T., & Ooi, T. (1989). Comparison of α-helix stability in peptides having a negatively or positively charged residue block attached either to the N- or C-terminus of an α-helix: The electrostatic contribution and anisotropic stability of the α-helix. *Biopolymers 28*, 995–1009.

Wada, A. (1976). The α-helix as an electric macro-dipole. *Adv. Biophys. 9*, 1–63.

Zimm, B.H. & Bragg, J.K. (1959). Theory of the phase transition between helix and random coil in polypeptide chains. *J. Chem. Phys. 31*, 526–535.