Ion-Pair and Charged Hydrogen-Bond Interactions between Histidine and Aspartate in a Peptide Helix[†]

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ABSTRACT: The effect on helix stability of placing a single pair of His−Asp or Asp−His residues, spaced (i, $i + 3$), (i, $i + 4$), or (i, $i + 5$), in an alanine-based peptide has been determined. The peptides have identical amino acid compositions, intrinsic helix propensities, and closely similar charge−helix dipole interactions, but they have different side chain interactions. Their helix contents are measured by circular dichroism over the pH range of 2−9, and the strength of a particular side chain interaction is determined from the increase in helix content over the reference peptide with the (i, $i + 5$) spacing. Side chain interactions are found for both the (i, $i + 3$) and (i, $i + 4$) spacings but only in the His−Asp orientation. Charged hydrogen-bond interactions occur at extreme pH values, and they are almost as strong as the ion-pair interactions at pH 5.5; but only the (i, $i + 4$) His−Asp peptide forms a strong hydrogen bond at pH 2, and only the (i, $i + 3$) peptide forms a strong hydrogen bond at pH 8.5. The ion-pair interactions are not screened effectively by 1 M NaCl, and hydrogen bonds probably acount for most of their strength.

Whether ion-pair interactions contribute favorably to protein stability is still under discussion. Although a His−Asp salt bridge (the term salt bridge is used here to denote a H-bonded ion pair) contributes −3 to −5 kcal/mol, or about one-half of the net stability of T4 lysozyme (Anderson et al., 1990), nevertheless, attempts to increase the stability of T4 lysozyme by making engineered His−Asp ion-pair interactions were unsuccessful (Dao-pin et al., 1991). Theoretical considerations suggest that the desolvation associated with burial or partial burial of charged groups should be unfavorable (Hendsch & Tidor, 1994; Honig & Nicholls, 1995) and earlier studies suggest that ion-pair interactions in proteins are strong interactions only when the interacting groups are partly buried (Friend & Gurd, 1979; Matthew & Richards, 1982; States & Karplus, 1987). Replacing three buried charged residues of Arc repressor with nonpolar residues does give increased stability [Waldburger et al., 1995; see also Hendsch et al. (1996)]. The role of ion-pair interactions in stabilizing coiled-coil dimer helices is also controversial (Krylov et al., 1994; Lumb & Kim, 1996; Lavigne et al., 1996, and references therein). The structures of some proteins from extreme thermophiles show networks of ion-pair interactions, and there is considerable curiosity about the possible role of these interactions in stabilizing proteins at high temperatures [see review in Goldman (1995)].

Consequently, it is interesting to determine how effective ion-pair interactions are in stabilizing solvent-exposed peptide helices. A Glu 2−Arg 10 ion-pair interaction proved to be one of the sources of the surprising stability of the C-peptide helix from the N-terminal end of ribonuclease A [see Fairman et al. (1990) and references therein]. Repeated blocks of Glu_4Lys_4 induce helix formation in a peptide when, in a peptide of the same amino acid composition, repeated blocks of Glu_2Lys_2 do not (Lyu et al., 1992), and the strength of the (i, $i + 4$) Glu−Lys ion-pair interaction was estimated at −0.5 kcal/mol. A system for measuring the strength of a specified ion-pair interaction based on placing a single pair of charged residues in an otherwise neutral Gln−Ala peptide helix was developed by Scholtz et al. (1993). They applied the method to Glu−Lys interactions and found a small (−0.4 kcal/mol) interaction in both the (i, $i + 3$) and (i, $i + 4$) spacings and with both the Glu−Lys and Lys−Glu orientations. In the studyof ion-pair interactions, it is necessary to take account of the "charge−helix dipole" interaction made by a charged residue, a term used here as an abbreviation for the electrostatic interactions made between a charged side chain and the partial charges on the backbone NH and CO groups of the peptide. Scholtz et al. (1993) used a highly simplified analytical representation of the charge−helix dipole interaction and then calibrated it; here we take approximate account of the effect by using a peptide with an (i, $i + 5$) spacing as a reference peptide [see Huyghues-Despointes et al. (1995)].

The ion-pair and charged H-bond[1] interactions made by His and Asp in alanine-based peptides are interesting for various reasons. As explained above, they should be useful in understanding the contradictory results found with T4 lysozyme. His and Asp have short side chains, and they are likely to make stronger interactions than residues such as Glu, Lys, or Arg, both because there is less loss of side chain entropy upon fixing an interaction and because, in proteins, His and Asp are more likely to be partly buried. Moreover, both residues titrate in the pH range of 2−9, and so their ion-pair interactions can be compared with any

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[1] Abbreviations: CD, circular dichroism; NMR, nuclear magnetic resonance; Asp, aspartate; H-bond, hydrogen bond; TSP, 3-(trimethylsilyl)propionic acid.

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charged H-bond interactions formed at pH 2 or 9. The pK_a values of His and Asp can be determined readily by NMR, by monitoring the chemical shifts of the Asp β CH_2 and the His ϵ1 CH protons.

EXPERIMENTAL PROCEDURES

Synthesis of Peptides, Purification, and CD Measurements. Peptides were synthesized by solid phase methods using an active ester coupling procedure, employing pentafluorophenyl esters of 9-fluorenylmethyloxycarbonyl (Fmoc)-unlabeled amino acids (Atherton & Sheppard, 1985), purchased from Milligen/Millipore. Peptides were cleaved and purified as described by Chakrabartty et al. (1991), and peptide purity was assessed by C18 reverse phase chromatography using a Pharmacia FPLC system. The parent-ion molecular weight of each peptide was confirmed by fast atom bombardment mass spectrometry (FAB). The conditions of storage and handling of the peptides were as described by Huyghues-Despointes et al. (1993a,b).

Concentrations of the peptide stock solutions were determined by measuring tyrosine absorbance in water (ϵ_{275} = 1390 M^{-1} cm^{-1}) (Brandts & Kaplan, 1973). Samples were prepared by diluting the aqueous stock solutions in 10 mM NaCl or 1.0 M NaCl buffered with 1 mM sodium citrate, 1 mM sodium phosphate, and 1 mM sodium borate prepared at either pH 2.0, 5.5, or 9.0. The pH dependence of helix content was measured by CD as described by Huyghues-Despointes et al. (1993a,b).

CD measurements were made with an AVIV 60 DS spectropolarimeter equipped with a Hewlett-Packard model 89100A temperature controller. The ellipticity was measured as mean residue ellipticity, [Θ] (deg cm^2 $dmol^{-1}$), and was calibrated with (+)-10-camphorsulfonic acid. Cuvettes with either a 1 mm or 1 cm path length were used. The degree of helical structure in each peptide was determined by monitoring the ellipticity at 222 nm at 0 °C. These peptides have properties similar to those of other monomeric alanine-based peptide helices [for review, see Scholtz and Baldwin (1992)]. Thermal unfolding curves are coincident at two different peptide concentrations that differ by 10-fold, indicating that helix formation is not caused by peptide association.

NMR Experiments. All NMR spectra were recorded at 2 °C on a 500 MHz General Electric GN-Omega spectrometer, using a 6000 Hz spectral width for one-dimensional data collection. Spectra were processed on a Silicon Graphics Personal Iris and Indigo computers using FELIX 2.3 (Hare Research, Inc.). Samples for pH titration were prepared by making a 1−2 mM peptide solution containing 10 mM NaCl and 3 mM sodium phosphate in D_2O. Peptide solutions for pH titration also included 1 mM TSP as a chemical shift standard. The free induction decay was the sum of 64 scans consisting of 4096 complex points. The pH dependence of the chemical shift of the β-methylene protons of aspartate and a histidine ring proton was measured relative to the standard, and taking account of the pH dependence of the chemical shift of TSP (De Marco, 1977). The observed pK_a values were determined by fitting the change in chemical shift with pH to the Henderson−Hasselbach equation.

Analysis of the Energetics. Helix contents were calculated from the ellipticities at 222 nm for each peptide by using the relationship $f_H = ([\Theta]_{obs} - [\Theta]_c)/([\Theta]_H - [\Theta]_c)$, where $[\Theta]_H = -42500(1 - 2.5/n)$ and $[\Theta]_c = 640$ deg cm^2 $dmol^{-1}$ at 0 °C (Scholtz et al., 1991; Rohl et al., 1996). The length of the peptide sequence is 18 for all peptides studied here. The standard Gibbs free energy, $\Delta G°$, of the side chain interaction was determined by using a modified version of the Lifson−Roig theory (Lifson & Roig, 1961; Stapley et al., 1995). The free energy of the side chain interaction is calculated by comparing the helix contents of the reference peptide and the peptide containing a side chain interaction. The reference and test peptides are treated by assigning an average intrinsic helix propensity, $\langle w \rangle_{host}$, to alanine and lysine residues. The other residues are given previously determined w and n (n-cap) values (Armstrong & Baldwin, 1993; Huyghues-Despointes et al., 1993a; Doig et al., 1994), which have been revised recently by Rohl et al. (1996) (w_{H^0} = 0.36, n = 2.12; w_{H^+} = 0.22, n = 1.0; w_D− = 0.38, n = 6.6; w_{D^0} = 0.40, n = 1.0; w_G = 0.048, n = 3.9; w_Y = 0.48, n = 4.9). The helix nucleation constant v^2 (=0.0013) is used for all residues (Rohl et al., 1992, 1996; Scholtz et al., 1991). p is defined as the equilibrium constant of the interaction in a completely helical peptide. The free energy of the side chain interaction is equal to $-RT \ln p$. The interaction is included in the partition function for all conformations in which the interacting residues and intervening residues are in a helical conformation, as defined by their (ϕ, φ) angles. The p value is adjusted until the observed and calculated helix contents for the test peptide agree.

Table 1: Peptide Sequences

peptide	sequence
HD3	Ac-KAAAA**H**AA**D**AAAAAAKGY-NH_2
HD4	Ac-KAAAA**H**AAA**D**AAAAAKGY-NH_2
HD5	Ac-KAAAA**H**AAAA**D**AAAAKGY-NH_2
DH3	Ac-KAAAAAA**D**AA**H**AAAAKGY-NH_2
DH4	Ac-KAAAAA**D**AAA**H**AAAAKGY-NH_2
DH5	Ac-KAAAA**D**AAAA**H**AAAAKGY-NH_2

RESULTS

Peptide Design. The effects of His−Asp interactions on helix stability cannot be analyzed using the neutral AQ host sequence, Ac-$(AAQAA)_3$Y-NH_2, used previously to measure other polar side chain interactions (Scholtz et al., 1993; Huyghues-Despointes et al., 1993a, 1995) because the peptides containing these residues are not very soluble in water and have very low helix contents. Instead, we use a new peptide design. The two reference sequences, shown in Table 1, have an (i, i + 5) spacing between His and Asp, and the sequences contain chiefly alanine residues to promote helix formation. Lysine residues are placed at the ends of the sequence to solubilize the peptide in water. The histidine and aspartate residues near the middle of the peptide also help to solubilize the peptide in water. In the pH range used for this study (pH 2−9), there are always at least three charged residues in each peptide. All peptides are 18 residues in length, have the N and C termini capped with an acetyl and an amide group to prevent unfavorable charge−helix dipole interactions, and have a tyrosine at the C

terminus for concentration determination. A glycine residue separates the tyrosine from the rest of the sequence to eliminate the contribution of the aromatic side chain to the far-UV CD spectrum (Chakrabartty et al., 1993).

There are two sets of peptides (HD or DH). One set (HD) has the histidine N-terminal to the aspartate, while the DH set has these residues in the opposite orientation. Each set consists of three peptides with the histidine and aspartate residues spaced either (i, $i + 3$), (i, $i + 4$), or (i, $i + 5$). The histidine is located in the same position within each set (position 6 in the HD set and position 11 in the DH set), and the aspartate is moved to change the spacing between the His−Asp residues. The interaction of the the helix dipole with aspartate should vary only slightly in the three peptides. The charge−helix dipole interaction diminishes as the aspartate moves closer to the center of the sequence (Huyghues-Despointes et al., 1993a).

Detection of Side Chain Interactions. The following rule is used here to detect side chain interactions. The helix content of a test peptide, with an (i, $i + 3$) or (i, $i + 4$) spacing, should be significantly greater than that of the reference (i, $i + 5$) peptide, and the difference should be larger than can reasonably be attributed to position effects. Peptide helix contents can be measured by CD with a reproducibility of ±3%; both the CD intensity and the peptide concentration enter into the determination of helix content [see Chakrabartty et al. (1993, 1994)]. A difference in helix contents of less than 9% is not considered significant. The position effects are discussed below. They are larger for His than for Asp, and consequently, the His position is kept fixed in each set of three peptides.

The first position effect is the frayed-end effect. A helix-breaking residue diminishes the helix content of a peptide more when placed near the center than near either end of a peptide, because the ends of the helix are partly frayed (Chakrabartty et al., 1991). The second position effect is the charge−helix dipole effect, which is an electrostatic interaction and may either stabilize or destabilize the helix, depending on whether it is attractive or repulsive. This effect is largest near the ends of the peptide. It has been measured both for Asp (Huyghues-Despointes et al., 1993a) and for His (Armstrong & Baldwin, 1993) by varying the position of a single charged residue throughout the length of an otherwise neutral Gln−Ala peptide. The third position effect arises from the Coulombic interaction among charged residues, which changes when the position of one charged residue is varied; usually, this effect is small compared to the other two effects.

The position effects seen here can be understood most simply by examining the behavior in Figure 1 of the two reference peptides HD5 and DH5, where the position effects are not complicated by side chain interactions. At pH 5.5, the helix content of DH5 is nearly twice that of HD5. The main cause is the difference in the side chain−helix dipole interactions, which are favorable in DH5 but unfavorable in HD5. Asp^- makes a favorable helix dipole interaction when it is close to the N terminus, while His^+ makes a favorable interaction when it is near the C terminus. The frayed-end effect may also make a small contribution to the difference between HD5 and DH5. His^+ has a lower helix propensity ($w = 0.22$) than Asp^- ($w = 0.38$); in DH5, His^+ is eight residues away from the C-terminal residue, while in HD5, His^+ is only six residues away from the N terminus. On the other hand, the penultimate glycine residue in these peptides effectively terminates the helix so that His^+ is nearly the same distance from an end of the helix in DH5 as in HD5.

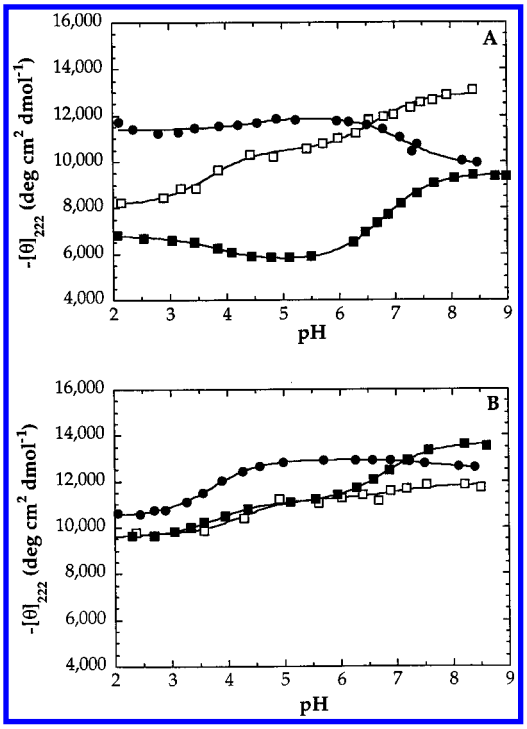

FIGURE 1: pH dependence of the helix content in the HD (A) and DH (B) set of peptides in 10 mM NaCl at 0 °C: (i, $i + 3$) (□), (i, $i + 4$) (●), and (i, $i + 5$) (■).

The effects of pH on the helix contents of HD5 and DH5 can be understood as follows. Peptide HD5 shows a large increase in helix content from pH 5.5 to 8.5 as $His^+ \rightarrow His^0$, whereas DH5 shows a smaller increase. Two effects contribute. The frayed-end effect decreases as $His^+ \rightarrow His^0$ because His^+ has a lower helix propensity ($w = 0.22$) than His^0 ($w = 0.36$), and the charge−helix dipole interaction is abolished as $His^+ \rightarrow His^0$. In HD5, the two effects are added, while in DH5, they partially cancel each other; the frayed-end effect is larger. In going from pH 5.5 to 2.0, as $Asp^- \rightarrow Asp^0$, there is a small decrease in the helix content of HD5 and a small increase in that of DH5. These effects are both caused by abolishing the charge−helix dipole interaction of Asp. The frayed-end effect is very small because the helix propensities of Asp^- ($w = 0.38$) and Asp^0 ($w = 0.40$) are nearly equal.

Considering first the percent differences in helix content between the test peptides and reference peptides, we conclude that (i) both HD3 and HD4 show H^+D^- ion-pair interactions at pH 5.5; (ii) HD4, but not HD3, shows a strong charged H-bond interaction (H^+D^0) at pH 2; (iii) HD3, but not HD4, shows a strong charged H bond (H^0D^-) at pH 8.5; and (iv) only marginal interactions of any kind are seen for the DH peptides. At pH 2, HD3 shows a marginal charged H-bond interaction. Considering next the possibility that position effects might account for some of these differences in helix content, we note that the helix content of the reference peptide DH5 is greater than that of either test peptide at pH 8.5, in contrast to the model used here for detecting side chain interactions. The magnitude of this effect sets a lower limit on the strengths of the interactions that can be measured by the method used here. We conclude that any marginal

Table 2: Energetics of His−Asp Interaction in 10 mM NaCl at 0 °C

	peptide	$-[\theta]_{222} \times 10^3$ (deg cm^2 dmol^{-1})	$f_{H(obs)}$[a]	$f_{H(ref)}$[b]	p[c]	ΔG (kcal/mol) per interaction
pH 2	HD3	8.2	0.24	0.17		
	HD4	10.8	0.31	0.17	2.9	−0.57
	HD5	5.9	0.18			
	DH3	9.4	0.27			
	DH4	9.6	0.27			
	DH5	9.5	0.27			
pH 5.5	HD3	10.6	0.30	0.18	3.1	−0.61
	HD4	12.0	0.34	0.17	4.0	−0.75
	HD5	5.6	0.17			
	DH3	10.8	0.31			
	DH4	12.9	0.36			
	DH5	11.3	0.32			
pH 9.0	HD3	13.0	0.37	0.25	2.3	−0.45
	HD4	9.6	0.27			
	HD5	8.7	0.25			
	DH3	11.3	0.32			
	DH4	12.0	0.34			
	DH5	13.3	0.37			

[a] The fraction of helix content calculated from the measured ellipticity by CD. [b] The fraction of helix content expected if the effect of a side chain interaction is excluded. [c] The p value is the equilibrium constant of a side chain interaction in a completely helical peptide.

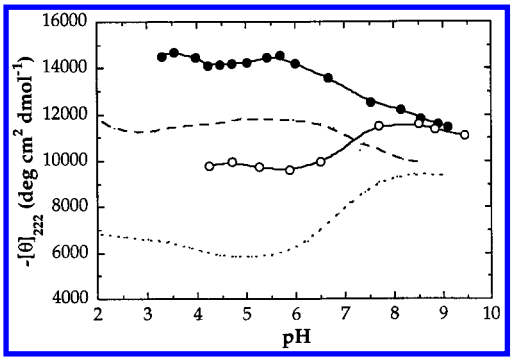

FIGURE 2: Comparison of the pH dependence of the helix content for HD4 (●) and HD5 (○) in 1.0 M NaCl versus 10 mM NaCl for HD4 (− − −) and HD5 (- - -).

interactions cannot be considered significant and that the significant side chain interactions observed here would not be considered significant, as measured by this method, if they were only $^1/_3$ as strong.

The strength ($\Delta G°$) of each interaction has been calculated as described in Experimental Procedures, and the values are given in Table 2.

Salt Dependences. In order to know if the ion-pair (H^+D^-) interactions shown in Figure 1A are screened effectively by 1 M NaCl, the helix contents of HD4 and HD5 and their pH dependences have been measured in 1 M NaCl (Figure 2). The results show that the difference in helix content between HD4 and HD5 is reduced only by 25% in 1 M NaCl. The probable conclusion is that an H bond accounts for most of the strength of each H^+D^- interaction and the H bond is not screened by 1 M NaCl; i.e., these H^+D^- interactions are salt bridge interactions. The NaCl screening studies of Huyghues-Despointes et al. (1993a,b) are best interpreted today by assuming that H-bond interactions are not screened detectably even by 4.8 M NaCl. The suggestion that H bonding accounts for most of the strength of the H^+D^- interactions is supported by observing strong charged H-bond interactions, at pH 2 for HD4 and at pH 8.5 for HD3.

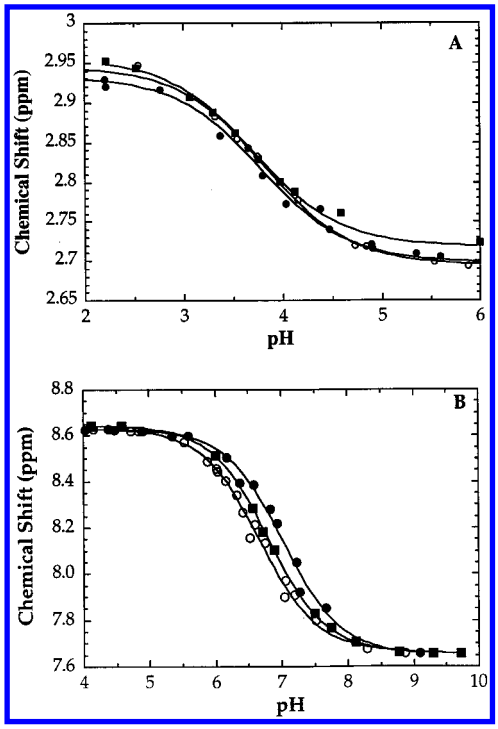

FIGURE 3: Dependence of the chemical shift of the His and Asp side chain proton resonances on pH: HD3 (■), HD4 (●), and HD5 (○).

Table 3: pK_a Values for Histidine and Aspartate in the HD Peptides

peptide	His ϵ1 CH proton	Asp β CH_2 protons
HD3	6.81	3.71
HD4	7.03	3.79
HD5	6.67	3.82

pK_a Shifts. When a two-state unfolding reaction such as protein unfolding is observed, the $\Delta G°$ of an ion-pair interaction can be determined equally well by pH titration of the native protein, and observing the shifts in the pK_a values of the interacting residues, as by breaking the interaction through mutation, and then determining the effect of the mutation on the $\Delta G°$ of unfolding [see Anderson et al. (1990)]. It is more difficult to use pK_a shifts in studying ion-pair interactions in peptide helices, because the helix−coil transition is a multistate reaction and the pK_a shifts are small.

The pK_a values of the His and Asp residues were determined in peptides HD3, HD4, and HD5 from the changes in chemical shift of the Asp β CH_2 protons and of the His ϵ1 CH proton. The results are given in Figure 3 and Table 3. The His pK_a of HD4 shows an increase of 0.35 pH init over that of HD5. This pK_a increase cannot be compared directly with the $\Delta G°$ of the interaction given in Table 2 because the $\Delta G°$ refers to the strength of the interaction in a completely helical peptide, whereas the pK_a is measured directly on the mixture of partly helical and random coil molecules. Peptide HD3 shows only a small increase in His pK_a (0.14 pH unit); the smaller increase is expected because HD3 makes a strong charged H-bond interaction (H^0D^-) at pH 8.5 and the shift in the His pK_a depends on the difference between the strength of the His−Asp interaction at pH 5.5 and 8.5.

The aspartate pK_a shifts (Table 3) are quite small. This behavior is expected for HD4, which makes a strong charged

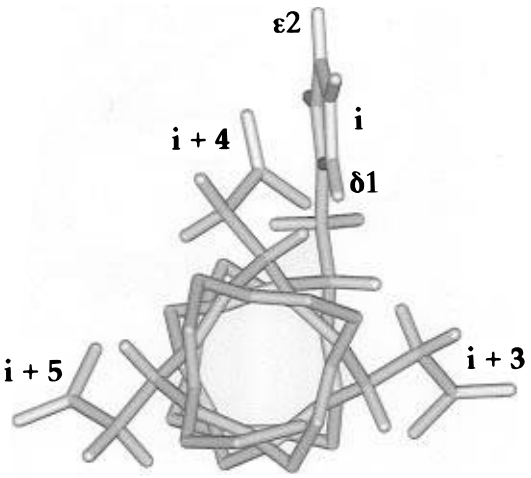

FIGURE 4: Comparison of His and Asp residues spaced (i, i+ 3) and (i, i + 4) in a model helix. The *trans* (His) and *gauche* + (Asp) χ_1 rotamers are shown.

H-bond interaction (H^+D^0) at pH 2, but HD3 also shows only a small pK_a shift and makes only a marginal H-bond interaction at pH 2.

DISCUSSION

Side Chain Interactions Based on H Bonds. His−Asp ion-pair interactions observed here evidently have specific geometries that are based on H bonds and the use of preferred side chain rotamers. (1) All interactions are observed only in the His−Asp orientation; they are not observed in the reverse Asp−His orientation. (2) A strong charged H-bond interaction with Asp neutral, at pH 2, is seen only with the (i, i + 4) spacing. (3) At pH 8.5, with His neutral, a charged H-bond interaction is seen only with the (i, i + 3) spacing. These specific effects, two of which clearly involve H bonding, imply that the ion-pair interactions also involve H bonds, i.e., that salt bridges are formed at pH 5.5. This suggestion is supported by the failure of 1 M NaCl to screen effectively the (i, i + 4) ion-pair interaction. Similar results were found by Scholtz et al. (1993) for Glu−Lys ion-pair interactions.

In the analysis of a problem of this kind, it is a major help when a side chain interaction found in a peptide helix occurs at an above-random frequency in protein helices and the structure of the interacting residue pair can be inferred from protein structures. This situation holds for the (i, i + 4) Gln−Asp H-bond interaction studied by Huyghues-Despointes et al. (1995), but (i, i + 4) and (i, i + 3) His−Asp residue pairs do not occur in protein helices with an above-random frequency (Klingler, 1996); the reason is not known. The reason why interacting His and Asp residue pairs do not occur in the reverse Asp−His orientation is very likely the same as in the Gln−Asp study. The *trans* χ_1 rotamer of Asp is strongly disfavored in the α-helix (McGregor et al., 1987), because the side chain CO group makes a close contact with a peptide CO group, and the structure of the interacting Gln−Asp pair has residue *i trans* and residue i + 4 *gauche* +. The reason why (i, i + 4) His−Asp makes a strong charged H bond at pH 2, whereas (i, i + 3) His−Asp does not, may be that the His and Asp side chains follow the general tendency of being closer in the (i, i + 4) spacing than in the (i, i + 3) spacing (see Figure 4). The reason for the unusual (i, i + 3) charged H-bond interaction at pH 8.5 is not known. A possible explanation lies in two distinct, nonsymmetrical proton donor sites, δ1 and ϵ2, of histidine. The pK_a of the δ1 proton is 0.6 pH unit lower than that of the ϵ2 proton (Creighton, 1993). In HD3, the preferred rotamer conformations may allow an interaction between the ϵ2 proton of histidine and aspartate that is either absent or much weaker in HD4.

Ion-Pair Interactions in Peptide Helices and Proteins. These results show that His−Asp ion-pair interactions occur spontaneously in peptide helices, and they are helix-stabilizing. The following question then arises. Why did the His−Asp ion-pair interactions engineered by Dao-pin et al. (1991) fail to stabilize T4 lysozyme? The answer may be that none of their His−Asp interactions was designed to occur within one helix; typically, they were designed to bridge two secondary structures. Substantial His pK_a shifts to higher values did occur, shifts as large as 1.0 pH unit (corresponding to $\Delta G° = -1.4$ kcal/mol). The pK_a shifts suggest that spontaneous ion-pair formation did occur, and the failure of these interactions to stabilize T4 lysozyme may have been the result of strain, just as engineered disulfide bonds in T4 lysozyme sometimes are destabilizing (Matsumura et al., 1989). The comparative ease of engineering charge−helix dipole interactions (Nicholson et al., 1991) that stabilize T4 lysozyme may be explained by the fact that they are electrostatic interactions without a requirement for H-bond formation.

Our results suggest that (i, i + 4) or (i, i + 3) His−Asp ion-pair interactions engineered within a protein helix should in some cases be stabilizing relative to the (i, i + 5) His−Asp residue pair made as a control. Our results suggest further that, in the controversy over whether ion pairs in proteins are stabilizing, a central issue is whether side chain H bonds are stabilizing in proteins [for a current review, see Myers and Pace (1996)].

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