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Charged histidine affects α-helix stability at all positions in the helix by interacting with the backbone charges

(helix dipole/side-chain–main-chain H-bond)

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ABSTRACT **To determine whether a charged histidine side chain affects α-helix stability only when histidine is close to one end of the helix or also when it is in the central region, we substitute a single histidine residue at many positions in two reference peptides and measure helix stability and histidine pK_a. The position of a charged histidine residue has a major effect on helix stability in 0.01 M NaCl: the helix content of a 17-residue peptide is 24% when histidine is at position 3 compared to 76% when it is at position 17. This dependence of helix content on histidine position decreases sharply in 1 M NaCl, as expected for counterion screening of the charge–helix dipole interaction. Results at interior positions indicate that the position of a charged histidine residue affects helix stability at these positions. Unexpectedly high values of the helix content are found when either neutral or charged histidine is at one of the last three C-terminal positions, suggesting that either form can stabilize an isolated helix by hydrogen bonding to a main-chain CO group.**

Interactions between charged side chains and the partial charges of the α-helix backbone (termed the helix dipole) are widely assumed to be important for helix stability only when the charged side chain is close to an end of the α-helix. The reason is that at either end of the helix there are uncompensated partial charges on peptide groups (positive charges on four NH groups at the N terminus and negative charges on four CO groups at the C terminus) that result from these groups not being H-bonded in the helix. The resulting charge distribution resembles a macrodipole (1, 2) with equal and opposite charges (+0.5, −0.5) close to either end of the helix. The existence of the macrodipole has been demonstrated by dipole moment measurements (1).

Calculations indicate that the interaction between a charged side chain and nearby peptide dipoles contributes to helix stability (3–6) and that these interactions may be important when the charged residue occupies interior positions in the helix (3, 4). Our aim is to test whether charged histidine affects helix stability only at positions close to one end of the helix or also in the middle. Numerous studies have been made of the effects of charged residues interacting with the helix dipole, both in isolated helices (7–10) and in protein helices (11–14), but in all these experiments the charged residues are close to an end of the helix.

To study this problem, we make two sets of 16- or 17-residue α-helical peptides with a single His residue substituted at various positions in the middle or near the ends of the helix. In the first series of AQH peptides, we use a reference peptide (15) that is electrically neutral, water soluble, and moderately helical. Because it has no extraneous charged residues, it is ideal for quantitative studies of electrostatic interactions. The second series of AKH peptides (16) has higher helix contents but contains three charged Lys residues. The helix content of the various peptides is measured using circular dichroism (CD), and the results are correlated with the position and the charge of the His residue. We use the terms "peptide helix content" and "helix stability" almost interchangeably because earlier work has shown that, when substitutions are made in a peptide and the number of residues is held constant, there is a direct relation among stability to unfolding by guanidinium chloride, thermal stability, and helix content at 0°C (17).

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MATERIALS AND METHODS

Peptides were synthesized and purified as described (18). pH titrations of helix content (see Fig. 1) were performed in 1 mM sodium citrate/1 mM sodium phosphate/1 mM sodium borate (CD buffer) with either 10 mM or 1 M NaCl in water at 0°C. To test whether the CD signal is independent of peptide concentration, CD is measured for samples that vary in concentration between 5 μM and 80 μM. A plot of millidegrees of ellipticity vs. peptide concentration yields a straight line with correlation coefficient 0.99 or better in all cases (data not shown). Stock solutions of AQH peptides were made freshly before use. Sample pH was adjusted using HCl and NaOH; measurement was made with an Orion pH meter calibrated with standard solutions at the temperature of the measurement. Peptide concentrations of stock solutions were measured by Tyr absorbance, either in H_2O or in 6 M guanidinium chloride, using Tyr extinction coefficients given (19).

The pK_a of His in two small unstructured peptides was determined using ^1H NMR. Samples contained ≈5 mM peptide, in CD buffer and 10 mM NaCl in 90% H_2O and 10% ^2H_2O. Spectra were obtained on a GE 500-MHz NMR spectrometer at 3°C and referenced to 3-(trimethylsilyl)propionic acid. The pH dependence of the chemical shift of 3-(trimethylsilyl)propionic acid was accounted for as described (20). The pK_a values were corrected to 0°C using the ionization enthalpy ΔH_i of His measured using the peptide Ac-AAHA-(NH_2). The ionization equilibrium constant was measured at 10°C increments between 0°C and 60°C by ^1H NMR. The resulting plot of the ionization constant K_i vs. $(1/T)$ (where T is temperature) gives a straight line (correlation coefficient = 0.997) with ΔH_i = 6.7 kcal/mol (1 cal = 4.184 J). This agrees with the previously measured value of 6.9 kcal/mol (21). The pK_a values at 0°C are shown in Fig. 3.

The fraction helix was calculated from the CD intensity at 222 nm as described (18). The pK_a values in Fig. 3 were obtained by fitting the pH titrations in Fig. 1 to the Henderson–Hasselbalch equation by using either one pK_a (for the AQH peptides) or two (for the AKH peptides) as described (22). Fitting the fraction helix data in Fig. 2 made use of the homopolymer approximation (18) in which the s values of Lys^+ and Ala are replaced by $\langle s \rangle$ = 1.47 (23) and the s values of Gln and Ala are replaced by $\langle s \rangle$ = 1.35 (24). A nucleation constant σ = 0.0030 was used. The nonlinear fitting program

NONLIN (25) was used. The Lifson–Roig theory (26) was used in fitting the data as described (18) and the Zimm–Bragg parameters σ and s (27) were computed from the corresponding Lifson–Roig parameters by using the relations given by Qian and Schellman (28). To fit the fraction helix data for charged His, the algorithm of Qian and coworkers (24) was used, in which the helix–random coil transition is described by the Lifson–Roig model and the backbone charges on each helical segment are approximated by a dipole with equal and opposite charges (+0.5, −0.5) at each end of the helical segment. The dielectric constant was taken as 60.

RESULTS AND DISCUSSION

Peptide Design and Properties. Two reference peptides are used here with the sequences Ac-$(AAQAA)_3$Y-NH_2 (15) (AQH peptides) and Ac-Y$(AAKAA)_3$A-NH_2 (16) (AKH peptides). A single Ala → His substitution is made, chiefly at positions that are not adjacent to Lys or Gln. The resulting peptides are named AQHX (AQ series) or AKHX (AK series) when His is substituted for Ala at position X. The peptides are designed to contain chiefly Ala because of the high helix propensity of Ala. The purpose of the Lys^+ or Gln residues is to solubilize the peptide in water. The two reference peptides are used to test the generality of the results.

The 17-residue AKH peptides are well behaved with regard to solubility and aggregation (22). The 16-residue AQH peptides are well behaved at CD concentrations if stock solutions are prepared freshly. CD spectra of both sets of peptides have minima at 208 and 222 nm and a maximum near 190 nm, characteristic of mixtures of α-helix and random coil. These peptides were made before it was discovered (29) that Tyr at the N or C terminus of a helical peptide makes a contribution from an aromatic band to the mean residue ellipticity at 222 nm. All peptides in a given series have a Tyr residue at the same position, and this effect does not influence the comparison of results from different peptides in the same series.

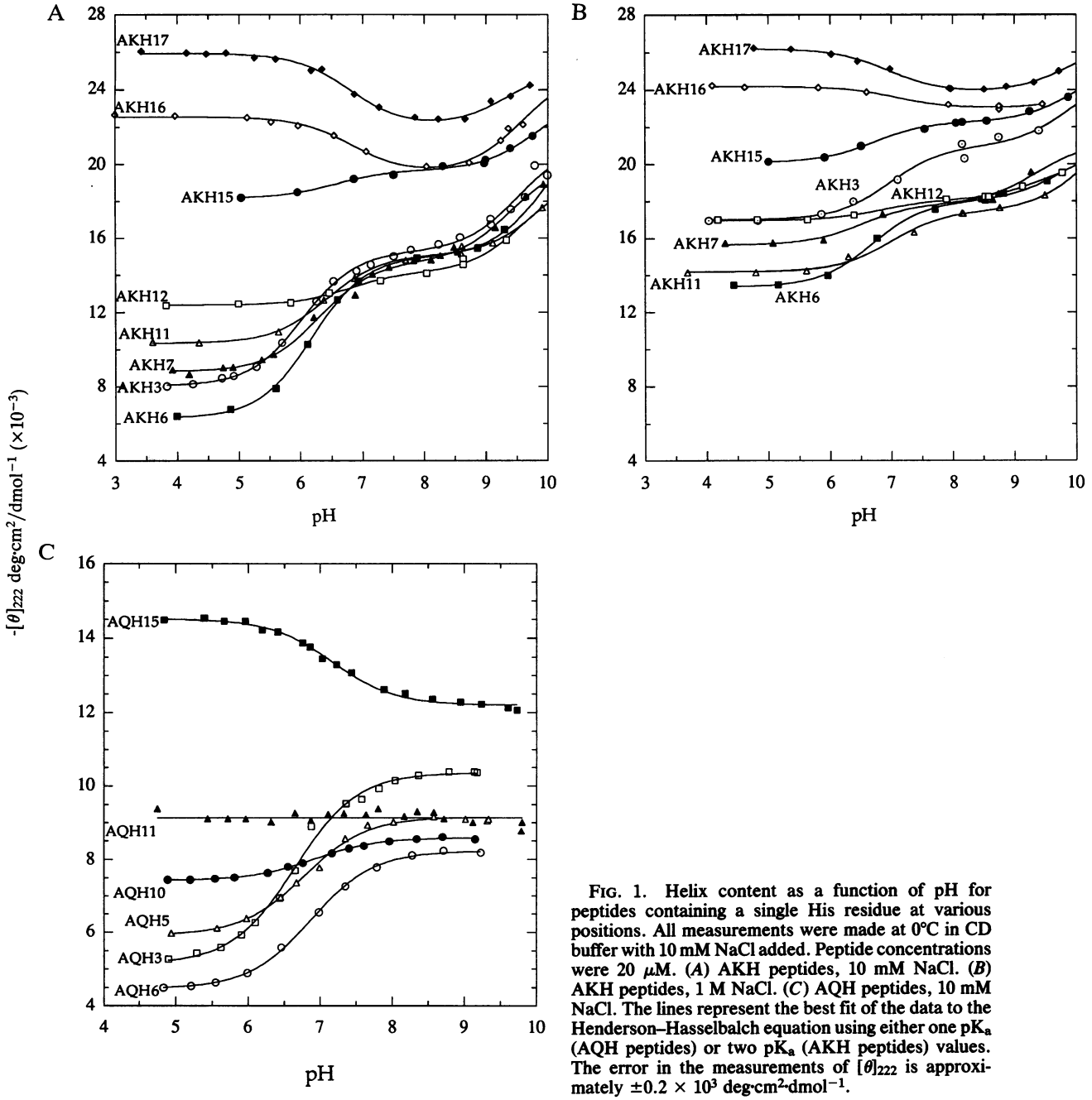

FIG. 1. Helix content as a function of pH for peptides containing a single His residue at various positions. All measurements were made at 0°C in CD buffer with 10 mM NaCl added. Peptide concentrations were 20 μM. (*A*) AKH peptides, 10 mM NaCl. (*B*) AKH peptides, 1 M NaCl. (*C*) AQH peptides, 10 mM NaCl. The lines represent the best fit of the data to the Henderson–Hasselbalch equation using either one pK_a (AQH peptides) or two pK_a (AKH peptides) values. The error in the measurements of $[\theta]_{222}$ is approximately $\pm 0.2 \times 10^3$ deg·cm²·dmol⁻¹.

Dependence of Helix Stability on the Position of a Charged His Residue. Fig. 1 shows that at low pH there is a striking effect on helix stability of the position of a charged His residue. We refer to this as the "position-dependence" effect. In Fig. 1*A* (AKH peptides, 0.01 M NaCl), the helix content varies from 24% to 76% as the His^+ position is changed from positions 3 to 17. The change in helix content decreases strongly in 1 M NaCl (Fig. 1*B*), indicating that a long-range electrostatic interaction is the primary factor, not H-bonding to the helix backbone. There are, however, two other effects that affect helix stability. One is helix stabilization by H-bonding of the His side chain to a main-chain CO group (see below). The other is the frayed-end effect, discussed below.

Fraying is predicted by helix-coil transition theory (26, 27) and is confirmed by substitution experiments in which a neutral helix-breaking residue (Gly) is substituted at various positions, either near one end or in the center of the helix, and the effect of substitution on the helix content of the peptide is measured (18). On the other hand, when the N-terminal α-NH_2 group is succinylated, so that the succinyl group can H-bond to a main-chain NH group, the N-terminal region is found to be helical by NMR criteria (10). Thus, it appears that a side-chain–main-chain interaction can block the fraying of a helix end substantially (10, 30).

Fraying of the helix ends affects the position dependence of a charged amino acid substitution in two ways. (*i*) If the charged residue is close to one end of the peptide and is within a frayed segment, it will interact less strongly with aligned charges in the helix backbone than if it is within a helical segment. (*ii*) Substitution of a helix-breaking residue close to one end of a helix has less effect on helix content than if it is near the center of the helix as shown by helix-coil theory and experiment (18). Both of these effects tend to diminish the influence of a charged helix-breaking residue on helix stability if the substitution is near a helix end. These effects explain why substituting His^+ at position 3 in the AK peptides destabilizes the helix less than does His^+ at position 6 (Fig. 1*A*) and likewise why the effect of His^+ is greater at position 6 than at position 2 in the AQ peptides (Fig. 1*C*).

Helix Stabilization by H-Bonding of His at Positions Near the C Terminus. The helix contents of peptides with a neutral His near the C terminus are unexpectedly high; compare results for the Ala → Gly substitutions at positions close to either end of the helix (18). Fig. 1*A* shows clearly that peptides AKH15–17 have higher helix contents than expected. H-bonding of a His side chain to a main-chain CO group near the C terminus of a helix has been observed both in wild-type barnase (11) and in His-substitution mutants (13) and it is likely that we are observing this effect here. Charged His also strongly stabilizes the helix when it is close to the C terminus in peptides AKH15–17 (Fig. 1 *A* and *B*) and AQH15 (Fig. 1*C*), and H-bonding to a main-chain CO group may be involved in addition to the charge interaction with backbone charges.

Helix Propensities (*s* Values) of Neutral and Charged His Residues. The curves of helix content vs. position of neutral His (His^0) have been jointly fitted to a single value of s (s = 0.36 at 0°C) for the AKH peptides (Fig. 2*A*) and AQH peptides (Fig. 2*B*). The fit is not closely determined, because of the H-bonding effect described above, and the range between the 67% confidence limits is $0.24 \leq s \leq 0.51$. Data points at positions close to the N terminus appear to be systematically low, for a reason not yet determined (AKH3 in Fig. 2*A* and AQH2 in Fig. 2*B*), and this effect also contributes to the uncertainty in s. The same effect has been seen in studies of Asp (31) and Glu (24) substitutions.

To fit the data for charged His, it is necessary to account for the charged side-chain–backbone interactions. This is done using the algorithm developed by Qian and colleagues (24). The estimated value of s for His^+ at 0°C is 0.06, and the range between 67% confidence limits is $0.01 \leq s \leq 0.13$. Thus His^+ is seen to be a strongly helix-breaking residue.

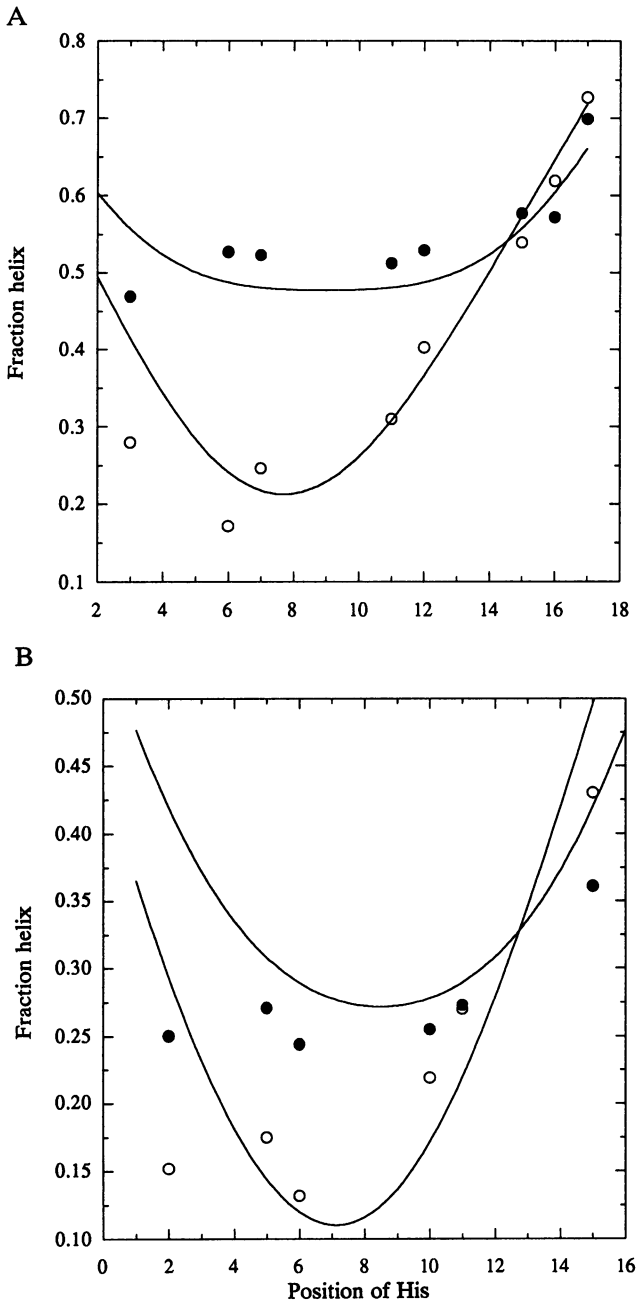

FIG. 2. Fraction helix vs. His position for peptides containing His^0 (solid symbols) and His^+ (open symbols). The fraction helix values were obtained from the fit of the data in Fig. 1 to the Henderson–Hasselbalch equation and are plotted vs. the position of the His substitution. (*A*) AKH peptides. (*B*) AQH peptides. The solid lines represent the fraction helix fitted for His^0 to the Lifson–Roig equation (18, 26) for the helix → random-coil transition and fitted for His^+ to an extended Lifson–Roig equation (24) that takes account of the charge–helix dipole interaction.

The values that we report here for s of His^0 and His^+ are substantially lower than the values found by the host–guest method using random-sequence copolymers of hydroxybutyl-L-glutamine and a guest (32). Similar large differences between s values determined with host–guest copolymers and with Ala-based peptides have been found for other amino acids and are discussed elsewhere (33, 34). Our s value for neutral His (0.36) is in good agreement with the value (0.37) as reported (34) for an Ala-based reference peptide.

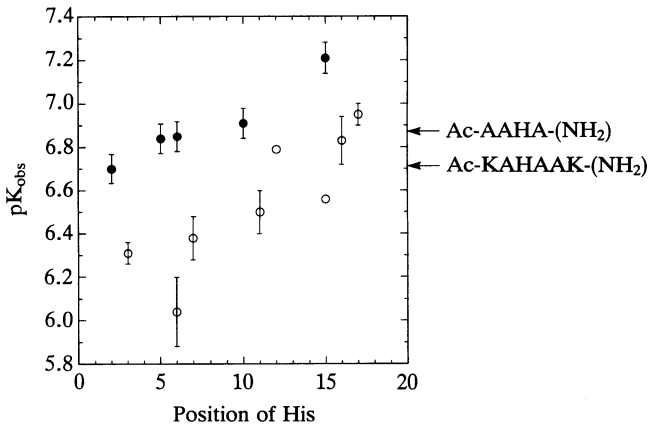

FIG. 3. Observed pK_a (pK_{obs}) of the His side chain in 10 mM NaCl, 0°C, derived from the data in Fig. 1 vs. the position of the His. Solid circles, AQH peptides; open circles, AKH peptides. The pK_a values of the short (nonhelical) peptides are indicated by arrows: Ac-KAHAAK-(NH_2), pK(His) = 6.72; Ac-AAHA-(NH_2), pK(His) = 6.88.

His pK_a at Interior Positions in the Helix. The most direct evidence for a His^+–backbone interaction that changes at interior positions in the helix comes from the increase in pK_a with position (Fig. 3). The shift in pK_a relative to an appropriate reference (e.g., the random coil) gives directly the standard Gibbs energy of the interaction (10), once account is taken of the fact that the molecule is not fully helical. In the random coil form, the pK_a is independent of position except for the influence of nearby charged groups. In the AQH peptides, there are no other charged groups except His^+.

Consequently, the observed increase in pK_a at positions between the N terminus and C terminus (Fig. 3) must mean that the charge–backbone interaction is changing significantly at these interior positions. The dependence of pK_a on interior position is not clear from the data on the AQH peptides alone. The increase in pK_a with position of the His residue should be stronger in a set of fully helical peptides, but we are unable to make such a set at present.

Concluding Remarks. The main objective of this work was to find out whether the interaction between charged His and backbone charges changes significantly at interior positions in the helix, as judged by its effect on helix stability and His pK_a. The results show that it does. Similar results have been found, after this work was begun, for Glu (24) and Asp (31) substitutions. Our results also reveal two other conclusions of interest. (*i*) H-bonding of the His side chain to a main-chain CO group near the C terminus, observed earlier in the protein barnase (11, 13), strikingly increases the stability of an isolated helix. (*ii*) Charged His has a remarkably low helix propensity (*s* value). We suggest that its low *s* value arises from a strong tendency to form a H-bond with a main-chain CO group at all positions in the helix. Thus these effects produce a remarkably strong dependence of helix stability on the position of a charged His residue.

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