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Nature of the charged-group effect on the stability of the C-peptide helix

(helix dipole/protein folding/protein electrostatics)

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ABSTRACT **The residues responsible for the pH-dependent stability of the helix formed by the isolated C-peptide (residues 1–13 of ribonuclease A) have been identified by chemical synthesis of analogues and measurement of their helix-forming properties. Each of the residues ionizing between pH 2 and pH 8 has been replaced separately by an uncharged residue. Protonation of Glu-2$^-$ is responsible for the sharp decrease in helix stability between pH 5 and pH 2, and deprotonation of His-12$^+$ causes a similar decrease between pH 5 and pH 8. Glu-9$^-$ is not needed for helix stability. The results cannot be explained by the Zimm–Bragg model and host–guest data for α-helix formation, which predict that the stability of the C-peptide helix should increase when Glu-2$^-$ is protonated or when His-12$^+$ is deprotonated. Moreover, histidine$^+$ is a strong helix-breaker in host–guest studies. In proteins, acidic and basic residues tend to occur at opposite ends of α-helices: acidic residues occur preferentially near the NH_2-terminal end and basic residues near the COOH-terminal end. A possible explanation, based on a helix dipole model, has been given [Blagdon, D. E. & Goodman, M. (1975) *Biopolymers* 14, 241–245]. Our results are consistent with the helix dipole model and they support the suggestion that the distribution of charged residues in protein helices reflects the helix-stabilizing propensity of those residues. Because Glu-9 is not needed for helix stability, a possible Glu-9$^-$···His-12$^+$ salt bridge does not contribute significantly to helix stability. The role of a possible Glu-2$^-$···Arg-10$^+$ salt bridge has not yet been evaluated. A charged-group effect on α-helix stability in water has also been observed in a different peptide system [Ihara, S., Ooi, T. & Takahashi, S. (1982) *Biopolymers* 21, 131–145]: block copolymers containing $(Ala)_{20}$ and $(Glu)_{20}$ show partial helix formation at low temperatures, pH 7.5, where the glutamic acid residues are ionized. $(Glu)_{20}(Ala)_{20}$Phe forms a helix that is markedly more stable than $(Ala)_{20}(Glu)_{20}$Phe. The results are consistent with a helix dipole model.**

Isolated C-peptide (residues 1–13 of RNase A, terminating in homoserine lactone at residue 13) shows partial α-helix formation in water near 0°C, conditions where C-peptide remains monomeric (1–3). This behavior is surprising because, as noted earlier (2), all short peptides (≤20 residues) are predicted not to show measurable α-helix formation in water, according to the Zimm–Bragg equation (4) and host–guest data (5) for helix formation. This point is illustrated in Fig. 1. To obtain 30% helix, which is the value shown by C-peptide at 0°C, pH 5.0, 0.1 M NaCl (2), Fig. 1 shows that $s \geq 1.7$ is required. But according to host–guest studies, all amino acids have s values near 1 (5). The amino acid residue in C-peptide with the largest host–guest value of s is phenylalanine with $s = 1.09$ (see Table 1). The Zimm–Bragg equation indicates that helix formation is not observable for a 13-residue peptide with $s = 1.09$ (Fig. 1). Consequently, there is a clear contradiction between the observation of partial helix formation by C-peptide and the prediction of the Zimm–Bragg equation, using host–guest values for the s and σ parameters.

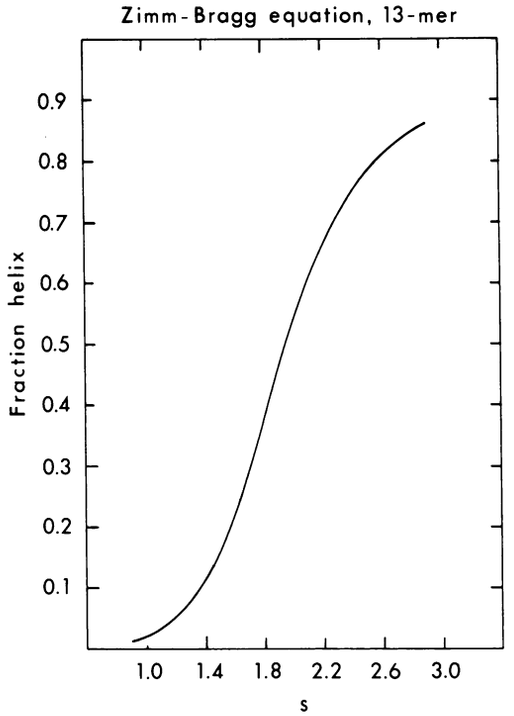

FIG. 1. The fraction of helix (θ_h, fraction of H-bonded residues) predicted by equation 3b of the Zimm–Bragg theory (4) for a 13-amino acid homopeptide (12 peptide units), using 8×10^{-4} for the helix nucleation constant σ [the host–guest value (6) for alanine]. θ_h is shown as a function of s, the helix stability constant. A simple approximation to the full equation used here was given earlier (2) for $n \leqq 20$, $\sigma \approx 10^{-4}$. Comparison with computer results for the full equation shows that the approximation gives θ_h within ≈1% error for $s \geqq 1.3$. For s values below 1.3 the approximation underestimates θ_h, and the estimate given earlier (2) for the predicted stability of the C-peptide helix (≈10^{-4}) is considerably too small. The correct value is shown in Fig. 1.

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Abbreviations: C-peptide, residues 1–13 of RNase A, terminating in homoserine lactone at residue 13; S-peptide-(1–15), residues 1–15 of RNase A; P-peptide, residues 1–8 of RNase A; RNase A, S, bovine pancreatic RNase A, S; δ, chemical shift.

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Charged groups play a special role in the formation of the C-peptide helix: stability of the helix is strongly pH dependent in the range pH 2–8 (2). Furthermore, the introduction of a single new charged group, an α-COO^- group at the COOH terminus, destabilizes the helix (3).

In this work, chemical synthesis of analogues is used to identify the residues responsible for the pH dependence of C-peptide helix stability. Each residue titrating between pH 2 and pH 8 has been replaced separately by an uncharged residue and helix stability has been measured as a function of pH. Earlier, a possible Glu-9^-···His-12^+ salt bridge was suggested as an explanation (2, 3) for both the pH dependence and the unexpected stability of the C-peptide helix. Recently, a possible Glu-2^-···Arg-10^+ salt bridge has been suggested as an additional stabilizing interaction (7). Our results are used to test the Glu-9^-···His-12^+ salt bridge and to find out if a salt bridge model can explain the charged-group effect.

Helix formation in the isolated S-peptide (residues 1–20) is localized in a manner resembling the intact protein. The helix stops near residue 13 in S-peptide (8, 9), and residue 13 is the last residue whose NH group is H-bonded in this helix in RNase A (bovine pancreatic RNase A).

MATERIALS AND METHODS

All peptides except [Leu^9]-S-peptide-(1–15) [S-peptide-(1–15) = residues 1–15 of RNase A] were synthesized by the solid-phase method (10) on the Stewart Mark V automatic synthesizer with chloroform as the solvent and trifluoracetic acid/chloroform (1:3) containing 0.1% indole for deprotection. COOH-terminal amides were synthesized on *p*-methylbenzhydrylamine poly(styrene/1% divinylbenzene) resin. Coupling reactions were monitored with the Kaiser test and repeated until complete, or were terminated by acetylation. Purification procedures included countercurrent distribution, reversed-phase partition chromatography on a C_8 silica gel, and preparative HPLC on a Bio-Rad large-pore (330 Å) reversed-phase C_4 HPLC column. Peptide purity was determined by high-voltage paper electrophoresis and by HPLC. Amino acid composition was confirmed by analysis on a Beckman 120C analyzer after hydrolysis in 6 M HCl at 110°C for 22 hr in the presence of mercaptoethanol and phenol. Purification procedures were chosen to minimize oxidation of Met-13 to the sulfoxide and hydrolysis of the side chain of Gln-11. In reference peptide II, these residues are replaced by alanine. The sequences of the peptides synthesized are given in Tables 1 and 2.

CD and NMR methods are described in preceding papers (2, 3, 9). CD spectra were recorded on a Jasco J-500A spectropolarimeter in the laboratory of J. T. Yang (University of California Medical School, San Francisco). Fourier-transform proton NMR spectra were recorded on a modified Bruker 360 MHz instrument at the Stanford Nuclear Magnetic Resonance Center. All samples contained 0.1 M NaCl. pH values in ^2H_2O solutions refer to uncorrected pH meter readings. Chemical shifts were corrected for the pH dependence of the chemical shift of the internal standard.

Chemically synthesized [Leu^9]-S-peptide-(1–15), prepared previously by the solid-phase method (11), was purified by ion-exchange chromatography (SP-Sephadex C-25) using a NaCl gradient (0.05–1.0 M) in 10 mM HCl; ion-exchange chromatography (SP-Sephadex C-25) using a 0–0.2 M NaCl gradient in 20 mM sodium phosphate (pH 7.5); and then gel filtration (Sephadex G-10) in 10 mM HCl. The purified peptide was judged to be >90% pure by NMR and amino acid analysis. Met-13, however, had been oxidized to methionine-sulfoxide; the ε-CH_3 resonance of methionine was a sharp singlet at the chemical shift δ = 2.72 ppm. Met-13 oxidation was not observed in the originally synthesized peptide (11) and may have occurred during storage or purification. The sulfoxide was reduced to methionine with *N*-methylmercaptoacetamide, kindly provided by C. H. Li, following the procedure of Houghten and Li (12). The peptide was dissolved in 5% (vol/vol) *N*-methylmercaptoacetamide and held under nitrogen for 72 hr at 37°C. Gel filtration (Sephadex G-15 in 10 mM HCl) was used to separate the peptide from reducing reagent. NMR analysis showed that reduction was >95% complete; the methionine ε-CH_3 resonance was now a singlet at δ = 2.08 ppm. The preparation of S-peptide-(1–15) from native S-peptide-(1–20), which follows the procedure of Potts *et al.* (13), has been described (14).

RESULTS

Two methods are used here to determine which residues are responsible for the charged-group effect. The first method (pH titration) is to synthesize a peptide in which only a single residue titrates in the pH region of interest, and then determine whether helix stability changes in parallel with titration of that residue. The second method (residue replacement) is to replace an ionizing residue with an uncharged residue and then measure the change in stability in favorable helix-forming conditions (pH 4.5, 3°C, 0.1 M NaCl).

Two reference peptides, I and II, are used: their amino acid sequences are given in Table 1. Reference peptide I was designed initially for testing the effect on helix stability of a possible Glu-2^-···His-5^+ salt bridge. It contains the substitution Lys-1 → acetylalanine because the two positive charges of Lys-1 might interfere with the formation of such a salt bridge. Reference peptide II contains Lys-1 and has the two substitutions Gln-11 → alanine and Met-13 → alanine because Gln-11 and Met-13 are the two troublesome residues in the synthesis and purification of C-peptide analogues (see *Materials and Methods*).

Reference peptides I and II are referred to as I and II. Substituted peptides are denoted by giving the reference peptide (I or II) and the substitution: II (Glu-9 → leucine) is reference peptide II with leucine in place of Glu-9.

His-12^+ Needed for Optimal Helix Stability. The pH titration method has been used with peptide I to determine

Table 1. Properties and sequences of C-peptide and the reference peptides I and II

Residue	C-peptide	Reference peptides I*	Reference peptides II*	pK†	s‡
1	Lys (2+)	Acetyl-Ala		7.6	0.94
2	Glu (−)			3.8	0.97
3	Thr				0.82
4	Ala				1.07
5	Ala				1.07
6	Ala				1.07
7	Lys (+)				0.94
8	Phe				1.09
9	Glu (−)			3.8	0.97
10	Arg (+)				1.03
11	Gln		Ala		0.98
12	His (+)			6.7	0.69
13	Hse-lactone	Met-$CONH_2$	Ala-$CONH_2$		—

*In the reference peptides I and II, amino acid differences from C-peptide are shown.

†The pK values were measured for S-peptide-(1–20) by ^1H NMR in ^2H_2O, 0.1 M NaCl; for Lys-1, the pK of the α-NH_3^+ group is given.

‡Host–guest values (5) of s (the helix stability constant) at 20°C and pH 7 for the amino acid residues in C-peptide; the values for lysine$^+$, glutamic acid$^-$, arginine$^+$ and histidine$^+$ are shown. The values of s for neutral glutamic acid and histidine are 1.35 and 0.85, respectively.

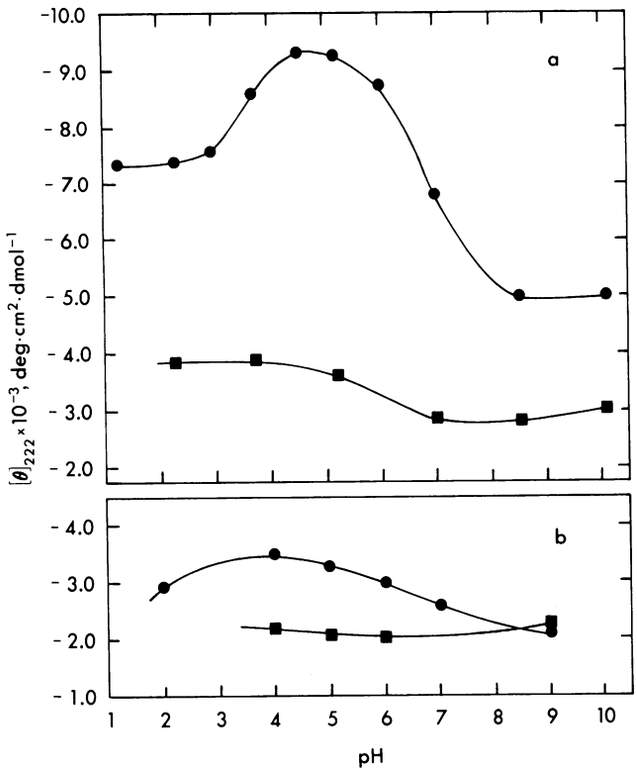

FIG. 2. pH dependence of mean residue ellipticity (222 nm) for reference peptide I (*a*) (see Table 1) and I (Ala-5 → histidine) (*b*) at 3°C (●), 0.1 M NaCl. The extent of helix formation is taken to be proportional to $-[\theta]_{222}$ after the baseline value determined at a high temperature (45°C, ■) is subtracted (2). A change in $-[\theta]_{222}$ of 2.65 × 10^4 deg·cm^2·dmol^{-1} has been estimated (2) for 100% helix formation by C-peptide if 10 peptide groups (those of residues 3–12) assume the α-helical conformation. The mean residue ellipticities given here should be increased by (13/10) before calculating the % helix in this way. Temperature dependence of ellipticity, in the absence of helix formation, is not taken into account in such an estimate.

whether His-12$^+$ is involved in the charged-group effect. The only group in I titrating between pH 5 and pH 8 is the side chain of His-12$^+$ (Table 1). Fig. 2*a* shows that the helix stability of I has an optimum near pH 5 and decreases sharply between pH 5 and pH 8. His-12$^+$ is thus identified as one of the residues responsible for the charged-group effect on the stability of the C-peptide helix.

The replacement method has been used to check this result. The peptide II (His-12 → alanine) does not show measurable helix formation in standard conditions (Table 2), confirming the result given by pH titration of I. Unfortunately, reference peptide II is itself not a strong helix-former (Table 2, Fig. 3*b*), and the decrease in helix stability caused by the substitution His-12 → alanine cannot be quantitated by use of these two peptides.

Glu-2$^-$ Needed for Optimal Helix Stability. The pH titration method has been used with II (Glu-9 → leucine) to study the role of Glu-2$^-$. In this peptide, which has a blocked α-COOH group (Table 1), Glu-2$^-$ is the only group titrating between pH 5 and pH 2. Fig. 3*a* shows a sharp decrease in helix stability between pH 5 and pH 2. Thus, Glu-2 is identified as the residue responsible for the acid branch of the pH-stability curve of C-peptide. Rico *et al.* (7) have also found recently that Glu-2$^-$ contributes to the stability of this helix.

The replacement method confirms this result. The peptide II (Glu-2 → alanine) shows no measurable helix formation in standard conditions (Table 2).

Glu-9$^-$ Not Needed for Helix Formation. An analogue of S-peptide-(1–15) with the substitution Glu-9$^-$ → leucine had been made earlier for studies of the folding of RNase S (11). Enough material remained to allow purification (*Materials and Methods*) and study. The peptide II (Glu-9 → leucine) was also synthesized here. Results with either peptide show that Glu-9$^-$ is not needed for C-peptide helix formation (Figs. 3*a* and 4*b*). Fig. 3*a* shows that the pH dependence of C-peptide helix stability between pH 2 and pH 5 is accounted for by titration of Glu-2$^-$. pH titration of δs, as shown in Fig. 4, has been used previously (2, 9) to demonstrate that residues throughout the helix show changes in δ as the helix is formed.

Table 2. Effects of amino acid substitutions on helix formation

Reference peptide	Charged group substitution	$[\theta]_{222}$ (3°C, pH 4.5, 0.1 M NaCl)
I	—	−9300
I	Ala-5 → His	−3400
II	—	−2800
II	Glu-9 → Leu	−5400
II	His-12 → Ala	0*†
II	Glu-2 → Ala	+1300*‡
P-peptide-(1–8)	—	+2100*§

Measurements of $[\theta]_{222}$ have been made at 45°C as well as at 3°C to check for temperature-dependent helix formation, as indicated in Figs. 2 and 3. In each case of partial helix formation at 3°C, the helix unfolds at 45°C, as reported for C-peptide lactone (2). Measurements of $[\theta]_{222}$ have also been made as a function of peptide concentration in the range 10–40 μM, and the values are independent of concentration. For the amino acid sequences of the two reference peptides, see Table 1. P-peptide, residues 1–8 of RNase A.
*CD spectrum indicates that peptide is not helical in 0.1 M NaCl.
†CD spectrum taken at 6°C.
‡CD spectrum taken at 2°C.
§CD spectrum taken at 4°C, pH 3.9.

The Substitution Ala-5 → Histidine Results in Decreased Helix Stability. Fig. 2 shows the effect on helix stability of the substitution Ala-5 → histidine. The peptide I (Ala-5 → histidine) is a weaker helix former than I over the pH range pH 2–8. This pair of peptides provides a test for the possible helix-stabilizing effect of a Glu-2$^-$···His-5$^+$ salt bridge. Such a salt bridge would be analogous to, and in addition to, a

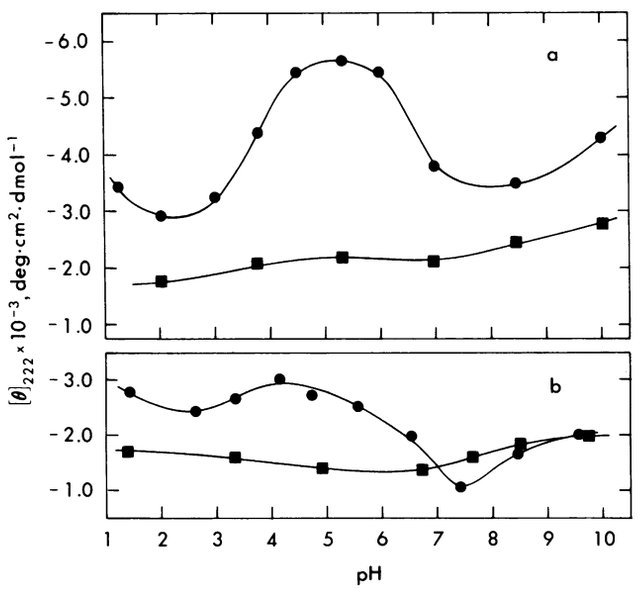

FIG. 3. pH dependence of mean residue ellipticity (222 nm) for reference peptide II (*b*) and II (Glu-9 → leucine) (*a*) at 3°C (●) and 45°C (■).

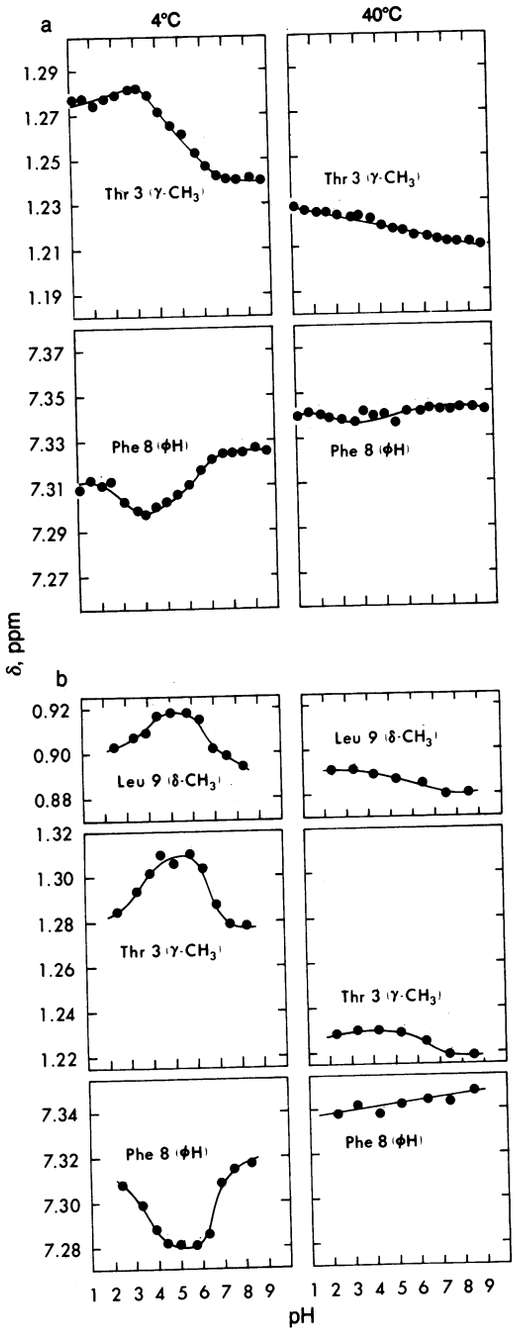

FIG. 4. pH dependence of δ for groups in S-peptide-(1–15) (*a*) and [Leu^9]-S-peptide-(1–15) (*b*) (^2H_2O, 0.1 M NaCl). δ changes in side chain resonances are used to monitor helix formation. Since the expected changes corresponding to 100% helix formation are not known, the changes with pH cannot be normalized. Instead, the test of whether or not all residues participate in a common structure-forming reaction (helix formation) is whether the δs change with pH in the same manner for all residues. To test for pH-dependent changes that are not caused by helix formation, the δs are also measured versus pH at a high temperature (40°C) where the helix is not present. The resonances measured are the γ CH_3 resonance of Thr-3 (average of two lines), the ring proton resonances of Phe-8 [average of three lines that shift with helix formation (2, 9)], and the δ CH_3 resonances of Leu-9 (average of four lines).

possible Glu-9$^-$···His-12$^+$ salt bridge postulated earlier (2).

Other experiments on helix formation by C-peptide analogues confirm that salt bridges of the type glutamic acid$^-$ (*i*)···histidine$^+$ (*i* + 3) do not significantly stabilize the helix (M. Dadlez, A. Bierzynski, M. Sobocinska, and G. Kupryszewski, personal communication).

DISCUSSION

Models for the Charged-Group Effect. The Zimm–Bragg model of α-helix formation makes the simplifying assumption that the *s* value of an amino acid residue is independent of its position in the helix. Our results demonstrate, however, that Glu-2$^-$ and Glu-9$^-$ have very different effects on the stability of the C-peptide helix. Position-dependent effects, not considered in the Zimm–Bragg treatment, apparently can be quite large.

Our results are not predicted from host–guest studies of α-helix formation in random copolymers (5) with hydroxypropyl- (or butyl-) L-glutamine as the host residue. In particular, host–guest data indicate that deprotonation of histidine$^+$ and protonation of glutamic acid$^-$ should increase helix stability, in contrast to our results for His-12$^+$ and Glu-2$^-$ in the C-peptide helix. Moreover, we find that His-12$^+$ is needed for optimal helix formation by C-peptide, whereas histidine$^+$ is a strong helix-breaker in host–guest experiments (5).

Salt-bridge model. Our results show that a Glu-9$^-$···His-12$^+$ salt bridge is not needed for helix formation because Glu-9$^-$ is not needed. In fact, the substitution Glu-9 → leucine results in increased helix stability, using either II or S-peptide-(1–15) as a reference peptide. The role of a possible Glu-2$^-$···Arg-10$^+$ salt bridge (7) remains to be studied.

Helix dipole model. The peptide bond has a substantial dipole moment and in an α-helix the peptide dipole moments add end-to-end across the H bonds to generate a macrodipole (15). Computer calculations of the field indicate that an extended line dipole gives a reasonable approximation for the field (16, 17). For a medium of low dielectric constant, the charge at each pole of the macrodipole is +0.5 or −0.5; the positive pole is near the NH_2 terminus and the negative pole is near the COOH terminus of an α-helix (16, 17). The interaction between a charged group and a nearby pole of the macrodipole will stabilize the helix if the two charges are of opposite sign and destabilize if they are of like sign. Calculation of the magnitude of this effect is, however, controversial (18).

The helix dipole model explains the charged-group effect in C-peptide as follows: (*i*) His-12$^+$ stabilizes the helix because it is close to the negative pole of the dipole; (*ii*) Glu-2$^-$ stabilizes the helix because it is close to the positive pole; and (*iii*) an α-COO$^-$ group at the COOH terminus of C-peptide destabilizes the helix (3) because it is close to the negative pole.

Results have been obtained in another peptide system that can be interpreted as strongly supporting the helix dipole model. Helix formation by $(Ala)_{20}$ in water also shows a charged-group effect when a block of charged residues at one end of the helix is used to solubilize $(Ala)_{20}$ (19). The copolymer $(Glu)_{20}(Ala)_{20}$Phe shows significantly more helix formation than $(Ala)_{20}(Glu)_{20}$Phe at pH 7.5, where the glutamic acid residues are ionized and poly-L-glutamate does not show helix formation. At 0.01 ionic strength, there is more than a 40°C difference in the melting temperatures of these two block copolymers (19). These results can be explained by a helix dipole model for the interaction of the $(Ala)_{20}$ helix with the charged glutamic acid$^-$ residues. Moreover, Takahashi and Ooi (S. Takahashi and T. Ooi, personal communication) have found that the position of a block of positively charged lysine$^+$ residues strongly affects the melting temperature of an $(Ala)_{20}$ helix. The position effect is opposite to that of a block of glutamic acid$^-$ residues.

The x-ray structures of proteins reveal some phenomena that have been interpreted in terms of interactions involving the helix dipole. Basic amino acids are found with high frequency at the COOH termini of α-helices and acidic amino acids are found preferentially at the NH_2 termini (20, 21). This distribution has been interpreted by Blagdon and Goodman

(21) as reflecting the helix-stabilizing character of the interaction between a charged group and an oppositely charged pole of the helix dipole. Phosphate-containing coenzymes are generally bound to the enzyme so that the phosphate group is close to the NH_2 terminus of an α-helix (16). The field arising from the positive pole of the helix dipole may aid in guiding the ligand to the correct site as well as providing one component of the binding free energy (16). α-Helices in all-helix proteins are generally arranged so that the helix dipoles are anti-parallel. This observation may mean (17, 22) that helix dipole–helix dipole interactions are important in stabilizing the folded structure. The crystal structure of a SO_4^{2-} binding protein shows that SO_4^{2-} is bound solely by 7 hydrogen bonds and sequestered from the solvent without any positively charged protein sidechains nearby (23). The SO_4^{2-} ion binds close to the NH_2 termini of three α-helices. Thus, the NH_2 terminus of a helix may be used to neutralize charge as well as to provide H-bond donor sites. The probable importance of the local peptide dipoles in binding the SO_4^{2-} ion has been pointed out (23).

Unexpected Stability of the C-Peptide Helix. Our results indicate that the charged-group effect on the stability of the C-peptide helix may be explained by a helix dipole model. The question then arises whether the helix dipole model can account for the entire difference between the observed and predicted stability of the C-peptide helix. This problem will have to be resolved in future work. Using a reference peptide that is a better helix-former than reference peptide II, it will be important to find out if partial helix formation can still be observed for an analogue of C-peptide in which the charged-group effect is absent.

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1. Brown, J. E. & Klee, W. A. (1971) *Biochemistry* **10,** 470–476.
2. Bierzynski, A., Kim, P. S. & Baldwin, R. L. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 2470–2474.
3. Kim, P. S., Bierzynski, A. & Baldwin, R. L. (1982) *J. Mol. Biol.* **162,** 187–199.
4. Zimm, B. H. & Bragg, J. K. (1959) *J. Chem. Phys.* **31,** 526–535.
5. Sueki, M., Lee, S., Powers, S. P., Denton, J. B., Konishi, Y. & Scheraga, H. A. (1984) *Macromolecules* **17,** 148–155.
6. Platzer, K. E. B., Ananthanarayan, V. S., Andreatta, R. H. & Scheraga, H. A. (1972) *Macromolecules* **5,** 177–197.
7. Rico, M., Gallego, E., Santoro, J., Bermejo, F. J., Nieto, J. L. & Herranz, J. (1984) *Biochem. Biophys. Res. Commun.* **123,** 757–763.
8. Rico, M., Nieto, J. L., Santoro, J., Bermejo, F. J., Herranz, J. & Gallego, E. (1983) *FEBS Lett.* **162,** 314–319.
9. Kim, P. S. & Baldwin, R. L. (1984) *Nature (London)* **307,** 329–334.
10. Stewart, J. M. & Young, J. D. (1984) *Solid Phase Peptide Synthesis* (Pierce Chemical, Rockford, IL).
11. Dunn, B. M. & Chaiken, I. M. (1975) *J. Mol. Biol.* **95,** 497–511.
12. Houghten, R. A. & Li, C. H. (1979) *Anal. Biochem.* **98,** 36–46.
13. Potts, J. T., Jr., Young, D. M. & Anfinsen, C. B. (1963) *J. Biol. Chem.* **238,** 2593–2594.
14. Kuwajima, K. & Baldwin, R. L. (1983) *J. Mol. Biol.* **169,** 281–297.
15. Wada, A. (1976) *Adv. Biophys.* **9,** 1–63.
16. Hol, W. G. J., van Duijnen, P. T. & Berendsen, H. J. C. (1978) *Nature (London)* **273,** 443–446.
17. Sheridan, R. P., Levy, R. M. & Salemme, F. R. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 4545–4549.
18. Rogers, N. K. & Sternberg, M. J. E. (1984) *J. Mol. Biol.* **174,** 527–542.
19. Ihara, S., Ooi, T. & Takahashi, S. (1982) *Biopolymers* **21,** 131–145.
20. Chou, P. Y. & Fasman, G. D. (1974) *Biochemistry* **13,** 211–221.
21. Blagdon, D. E. & Goodman, M. (1975) *Biopolymers* **14,** 241–245.
22. Hol, W. G. J., Halie, L. M. & Sander, C. (1981) *Nature (London)* **294,** 532–536.
23. Quiocho, F. A. & Pflugrath, J. W. (1985) *Nature (London)*, in press.