ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/19702607

The C-peptide Helix from Ribonuclease A Considered as an Autonomous Folding Unit

Article *in* Cold Spring Harbor Symposia on Quantitative Biology · February 1987

DOI: 10.1101/SQB.1987.052.01.045 · Source: PubMed

CITATIONS
36

READS
23

6 authors, including:

Robert Fairman
Haverford College
101 PUBLICATIONS **4,669** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

Writing papers View project

All content following this page was uploaded by Robert Fairman on 15 July 2015.

The user has requested enhancement of the downloaded file.

Downloaded from symposium.cshlp.org on March 7, 2012 - Published by Cold Spring Harbor Laboratory Press

Cold Spring Harbor Symposia on Quantitative Biology

The C-peptide Helix from Ribonuclease A Considered as an Autonomous Folding Unit

K.R. Shoemaker, R. Fairman, P.S. Kim, et al.

Cold Spring Harb Symp Quant Biol 1987 52: 391-398
Access the most recent version at doi:10.1101/SQB.1987.052.01.045

References This article cites 51 articles, 11 of which can be accessed free at:
http://symposium.cshlp.org/content/52/391.refs.html

Article cited in:
http://symposium.cshlp.org/content/52/391#related-urls

Email alerting service Receive free email alerts when new articles cite this article - sign up in the box at the top right corner of the article or **click here**

To subscribe to *Cold Spring Harbor Symposia on Quantitative Biology* go to:
http://symposium.cshlp.org/subscriptions

Copyright © 1987 Cold Spring Harbor Laboratory Press

Downloaded from symposium.cshlp.org on March 7, 2012 - Published by Cold Spring Harbor Laboratory Press

The C-peptide Helix from Ribonuclease A Considered as an Autonomous Folding Unit

K.R. SHOEMAKER, R. FAIRMAN, P.S. KIM,* E.J. YORK,† J.M. STEWART,† AND R.L. BALDWIN
Department of Biochemistry, Stanford University School of Medicine, Stanford, California 94305;
†*Department of Biochemistry, University of Colorado School of Medicine, Denver, Colorado 80262*

One of the basic goals of work on the mechanism of protein folding is to define autonomous folding units: those individual segments of a protein which, if excised, contain sufficient structural information to specify their own folding. Surprisingly little is known about this subject. A common opinion is that entire domains are the units of folding. A domain is defined as (1) a continuous segment of polypeptide chain that is folded on itself, as judged by inspection of the protein's X-ray structure, and (2) a folded segment that makes only marginal contacts with neighboring segments. Domains are usually found to contain 100–150 amino acid residues. The nature of protein structure is such that a domain can be divided into two subdomains and each subdomain can be further divided, if the definition of a subdomain is based on part 1 above (Rose 1979), without part 2.

Autonomous folding units might, however, prove to be substantially smaller than entire domains. Gilbert's hypothesis that new proteins can be made by reshuffling exons (Gilbert 1978, 1985) implies that each exon product is an autonomous folding unit (cf. Gō 1981) and the peptide segments encoded by exons are often as small as 30–40 residues. The definition of an autonomous folding unit is tied to the criteria used for stability of the folded structure. Our criteria for an autonomous folding unit are as follows: (1) The structure shown by the excised fragment should be sufficiently stable in aqueous solution to be detected, and should occur by a monomolecular reaction. (2) This structure must closely resemble the structure in the intact protein.

Other types of experiments on autonomous folding units may lead to alternative definitions. It seems likely that peptide reshuffling experiments, based on the methods of genetic engineering, will be used in future work to analyze autonomous folding units. Studies of sequence homology between limited regions of different proteins have been used to suggest folding units and to examine the role of exon boundaries in fixing these folding units (Doolittle 1985). Another approach to the analysis of folding units is to examine the ability of a protein fragment to bind a specific ligand such as heme (Craik et al. 1981), ATP (Knight and McEntee 1986; Barden and Kemp 1987), α-bungarotoxin (Wilson et al. 1985), or DNA (Bruist et al. 1987).

Autonomous folding units should be larger than pentapeptides, according to the results of Kabsch and Sander (1984), who find that a given pentapeptide sequence can occur as part of an α helix in one protein and part of a β sheet in a different protein. On the other hand, Wright and co-workers have reported formation of a β turn, a four-residue structure, within a nonapeptide fragment of hemagglutinin in aqueous solution (Dyson et al. 1985). Thus, sequences as short as β turns may be autonomous folding units. Only a small fraction of a protein may consist of autonomous folding units. At an early stage in the folding process, only a few segments may be folded and other residues may play a passive role.

Evidence That the C-peptide Helix Is an Autonomous Folding Unit

Residues 3–13 are helical in bovine pancreatic ribonuclease A (RNase A) and only a few of these residues bind the helix to the rest of the protein (Wlodawer and Sjölin 1983). Peptides containing this sequence show partial helix formation in aqueous solution (Brown and Klee 1971; Bierzynski et al. 1982; Kim and Baldwin 1984). The helix has been studied in C-peptide (residues 1–13 of RNase A, terminating in homoserine lactone at residue 13), in S-peptide (residues 1–20 of RNase A), and in chemically synthesized analogs of these peptides.

The following observations suggest that the C-peptide helix is an autonomous folding unit. (1) The C-peptide helix is much more stable than predicted by the Zimm-Bragg equation[1] when host-guest data on synthetic polypeptides (Sueki et al. 1984) are used to provide the Zimm-Bragg parameters. Consequently, the C-peptide helix may be stabilized by specific side-chain interactions and its side-chain structure may be similar to that of the 3–13 helix in RNase A. (2)

*Present address: Whitehead Institute, Nine Cambridge Center, Cambridge, Massachusetts 02142.

[1]Shoemaker et al. (1985) give the predicted stability of a 13-residue polyalanine helix and show that the C-peptide helix is about 30 times more stable than expected. They corrected an earlier estimate by Bierzynski et al. (1982), who used an approximate form of the Zimm-Bragg equation. Current studies of alanine peptides that are made water-soluble by inserting a few Lys and Glu residues show that these helices are also more stable than predicted from host-guest data (S. Marqusee and R.L. Baldwin, in prep.).

Downloaded from symposium.cshlp.org on March 7, 2012 - Published by Cold Spring Harbor Laboratory Press

Additional evidence that side-chain interactions stabilize the C-peptide helix is given by the strong pH dependence of helicity (Bierzynski et al. 1982). The curve of helicity versus pH, with a maximum at pH 5 and apparent pKs near 3.5 and 6.5, suggests that charged amino acid side chains are critical for helix stability. (3) The helix is localized in S-peptide and does not extend to the carboxy-terminal end, as shown by different types of NMR experiments (Rico et al. 1983; Kim and Baldwin 1984). There is a "helix-stop" signal in S-peptide whose nature remains to be determined. In synthetic polypeptides the average length of a helical sequence at the midpoint of the helix-coil transition is about 100 residues. Thus, localization of the helix in S-peptide gave the first definite indication that it is an autonomous folding unit.

Helix formation by isolated C-peptide occurs monomolecularly, as shown by molecular weight measurements (Brown and Klee 1971), by lack of concentration dependence (Bierzynski et al. 1982), and by gel filtration experiments (Shoemaker et al. 1987). Although the C-peptide helix is only marginally stable, helix formation can be studied readily near 0°C in the absence of organic cosolvents. Moreover, the helicity of C-peptide can be increased by certain amino acid substitutions without increasing the number of residues (Shoemaker et al. 1987).

Invariant Residues and Models for the Determination of the 3–13 Helix

Amino acid sequences for ribonucleases homologous to bovine pancreatic RNase A are known from 38 mammalian species. If we assume that residues 3–13 form the α helix in all 38 ribonucleases, then a study of the invariant residues and their roles in different helix prediction schemes might reveal features of the 3–13 helix involved in its behavior as an autonomous folding unit. The most striking feature of the invariant residues, which are shown in Figure 1, is their large number: in the sequence 1–15, Glu-2, Ala-5, Lys-7, Phe-8, Arg-10, Gln-11, His-12, and Asp-14 all are invariant and some other residues are replaced only by closely related amino acids. Reasons for the invariance of some of these residues have been proposed: His-12 is essential for catalytic activity; Gln-11 aids in substrate binding; Phe-8, His-12, and Asp-14 are needed for tight binding of S-peptide to S-protein (Blackburn and Moore 1982).

The relation between the invariant residues and the helix prediction schemes of Chou and Fasman (1974), Burgess et al. (1974), and Lim (1974) has been discussed by Lenstra et al. (1977). None of these schemes attempts to predict whether the 3–13 helix should be stable in C-peptide, and Lim's method is explicitly based on finding a cluster of side chains, when the peptide is in a helical conformation, that could bind the helix to the protein. Lenstra et al. (1977) suggest that the success of these different predictive schemes is closely correlated with the invariance of the hydrophobic residues. On the other hand, interactions involving ionizing groups contribute to the behavior of the 3–13 helix as an autonomous folding unit (see below). The problem of predicting from sequence which residues can form α helices that are autonomous folding units may be a separate problem from that of predicting α helices in proteins.

Based on studies of the C-peptide helix, we suggest the following model for α helices that are autonomous folding units: they are stabilized by specific intrahelical side-chain interactions which may function also to provide start and stop signals for localizing the helix. Other plausible mechanisms for start and stop signals can be suggested, notably the use of proline residues (Perutz et al. 1965). The concept that α helices might be stabilized by specific side-chain interactions was foreseen by Sela, Katchalski, and co-workers (Ramachandran et al. 1971; Schechter et al. 1971; Goren et al. 1979) who synthesized two types of peptides with simple repeating sequences in order to look for this effect. Maxfield and Scheraga (1975) found statistical evidence in protein α helices for an interaction between Glu and any basic amino acid four residues away (see also Sundaralingam et al. 1985).

Proposed Side-chain Interactions in the C-peptide Helix

In recent work, three types of specific side-chain interactions have been proposed to explain the stability of the C-peptide helix (Fig. 2). (1) Interaction between the α-helix macrodipole and charged groups near the ends of the helix was suggested by Shoemaker et al. (1985) after finding that Glu-2$^-$ and His-12$^+$ are the charged groups responsible for the pH dependence of C-peptide helicity. This interaction is studied in more detail in recent work (Shoemaker et al. 1987). (2) A H-bonded ion pair between Glu-2$^-$ and Arg-10$^+$ is

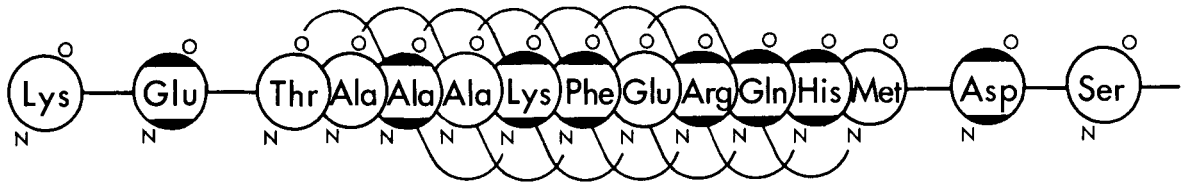

Figure 1. Sequence of the first 15 amino acids in bovine pancreatic RNase A. Darkened residues are invariant in ribonucleases from 38 mammalian species (Blackburn and Moore 1982). In 2 of 38 species, residue 11 is listed as either Gln or Glu with the other 36 as Gln, and also residue 14 is listed as either Asp or Asn in 2 species, with the other 36 as Asp.

Downloaded from symposium.cshlp.org on March 7, 2012 - Published by Cold Spring Harbor Laboratory Press

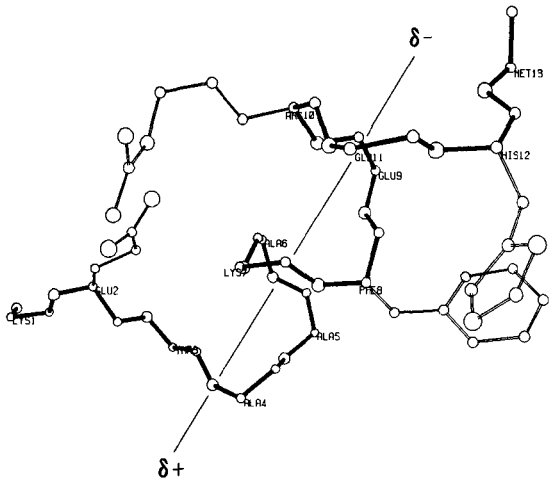

Figure 2. Residues 1–13 of RNase A illustrating the H-bonded ion pair between Glu-2$^-$ and Arg-10$^+$, a possible aromatic interaction between Phe-8 and His-12, and the α-helix macrodipole. All other side chains have been omitted for simplicity. The coordinates of RNase A are from Wlodawer and Sjölin (1983) and are deposited in the Brookhaven Protein Data Bank.

known to be present in RNase A (Wlodawer and Sjölin 1983), and it has been suggested that it should also be present in C-peptide and S-peptide (Rico et al. 1984, 1986; A. Bierzynski, pers. comm.). (3) Phe-8 and His-12 are positioned in RNase A so that they might make an aromatic interaction of the type discussed by Burley and Petsko (1985) and Blundell et al. (1986) for Tyr, Phe, and Trp. The existence of an aromatic interaction between Phe-8 and His-12 in C-peptide and S-peptide has been proposed (Bermejo et al. 1986; Bierzynski et al. 1986; Rico et al. 1986). It has been suggested further that His-12$^+$ interacts more strongly with Phe-8 than does His-12^0 (A. Bierzynski, pers. comm.), thereby providing a possible explanation for the effect of pH titration of His-12 on the helicity of C-peptide.

Interaction of the Helix Dipole with Charged Groups

The dipoles of the peptide groups add across H bonds so that the α helix has a large dipole moment that is proportional to helix length (3.4 Debye units per residue; Wada 1976). Calculations using point charges indicate that the electrostatic field around an α helix with no formal charges can be represented to a good first approximation by an extended line dipole whose positive and negative poles are near the amino and carboxyl termini, respectively (Hol et al. 1978; Sheridan et al. 1982). A more detailed picture is given by noting that four NH and four CO groups at the amino and carboxyl termini, respectively, of an α helix are not H-bonded and that the partial charges of the helix dipole actually reside on these NH and CO groups. Consequently, a negatively charged group near the amino terminus is helix-stabilizing, but becomes helix-destabilizing if placed near the carboxyl terminus, whereas the opposite is true of a positively charged group. In X-ray structures of proteins, there are many examples of negatively charged ligands bound close to the amino termini of α helices (Hol 1985). The distribution of charged groups in protein α helices is highly asymmetric, with acidic and basic residues found close to the amino and carboxyl termini, respectively (Chou and Fasman 1974, 1978), and this finding has been explained as reflecting the helix-stabilizing effects of these charged groups (Blagdon and Goodman 1975).

Recent experiments have shown that the charge on the amino-terminal residue of C-peptide affects the stability of the helix according to the qualitative predictions of the helix dipole model (Shoemaker et al. 1987). The naturally occurring residue, Lys-1, which has two positive charges, is helix-destabilizing relative to Ala, whereas succinyl-Ala-1, with one negative charge, is helix-stabilizing. Removing the positive charge on the α-NH_3^+ group of Ala-1 by pH titration is helix-stabilizing, whereas protonation of succinyl-Ala-1$^-$ is helix-destabilizing. Increasing the concentration of NaCl increases helicity for Lys-1^{++} and decreases helicity for succinyl-Ala-1$^-$, as expected for screening of charged-group interactions with the helix dipole.

In early work it was found that the α-COOH form of C-peptide shows much lower helicity than the lactone form (Kim et al. 1982; see also Rico et al. 1986). pH titration of the α-COOH group in C-peptide carboxylate affects the chemical shift of the amide proton of His-12, which is too far away to be affected by a through-bond interaction (Bundi and Wüthrich 1979), and the same effect was found in *N*-acetyl-Gly-His-Gly (Kim et al. 1982). They suggested that an ion pair is formed between the α-COO^- group and the protonated side chain of His-12 and that it shifts the helix-coil equilibrium toward the unfolded form. From the work of Shoemaker et al. (1987), it is evident that interaction of the α-COO^- group with the helix dipole offers an alternative explanation. To test this point, we synthesized the analog of C-peptide (peptide III[Glu-2 → Ala, Arg-10 → Ala, α-$CONH_2$ → α-COOH]) whose pH titration of helix content is shown in Figure 3. The peptide lacks Glu-2 so that only the α-COOH group is titrated between pH 2 and pH 5. As Figure 3 shows, protonation of the α-COO^- group is helix-stabilizing. Comparison of this peptide to one without a free α-COOH group does not involve comparing the carboxylate form versus the lactone form, so there is no question of whether the lactone stabilizes the helix by some other mechanism.

Next we reinvestigated the NMR evidence for an ion pair interaction between the α-COO^- group and the protonated side chain of His-12. Figure 4 shows pH titration of the chemical shifts of some protons in Gly-Gly-Phe-Ala. Titration of the α-COOH group between pH 2 and pH 5 causes substantial changes in the chemical shift of the Gly-2 αCH_2 resonance and of the Phe-3 $\beta,\beta' CH_2$ resonances. These groups are too far from the α-COOH group to experience through-bond effects (Bundi and Wüthrich 1979). Instead, the changes in chemical shifts apparently are caused by a ring current

Downloaded from symposium.cshlp.org on March 7, 2012 - Published by Cold Spring Harbor Laboratory Press

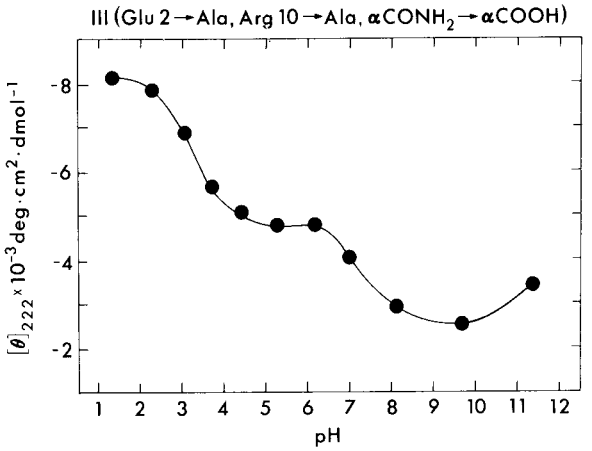

Figure 3. pH titration of helicity for peptide III(Glu-2→ Ala, Arg-10→ Ala, α-$CONH_2$→ α-COOH), which has only a free α-COOH group ionizing in the pH range 2–5. (The sequence of reference peptide III is given in Fig. 5 legend.) The figure shows an increase in helicity as the pH is reduced from 5 to 2 and the α-COO^- group is protonated. The effect of titrating His-12 (between pH 9 and pH 6) can also be seen. The mean residue ellipticity at 222 nm was measured at 3°C, 0.1 M NaCl. For experimental methods, see Shoemaker et al. (1985, 1987). The change in $[\theta]_{222}$ for complete helix formation by C-peptide at 3°C is near 30,000 deg cm^2 $dmol^{-1}$, when the baseline value for 0% helix is estimated as +3,000 deg cm^2 $dmol^{-1}$ (Mitchinson and Baldwin 1986; K.R. Shoemaker et al., in prep.).

effect. The rotamer populations for the Phe-3 αCH—$\beta,\beta'CH_2$ bond were calculated from measurements of the coupling constants and equations given by Kopple et al. (1973) and by Pachler (1964) and Feeney (1976), with similar results. The fraction of rotamer III (gauche-gauche) is small and independent of pH between 1 and 4.5 (f[III] = 0.08–0.09 according to Kopple et al. or 0.11–0.12 according to Pachler and Feeney), whereas in this pH range f(II) falls from 0.36 to 0.26 (Kopple et al. 1973) or to 0.27 (Pachler 1964 and Feeney 1976), and f(I) rises accordingly. The α-COOH group is farthest from the aromatic ring of Phe-3 in rotamer I.

For the peptide Gly-Gly-Phe-Ala, there cannot be an ion pair between the α-COO^- group and a neighboring side chain. We conclude that the ion pair explanation is also unlikely for *N*-acetyl-Gly-His-Gly, for which similar NMR results were obtained (Kim et al. 1982). Therefore, the NMR results for both peptides probably arise from a ring current effect that reflects the rotamer populations of the aromatic side chain (Phe or His). Having thus ruled out the His-$12^+ \cdots \alpha$-COO^- interaction, we conclude that the increase in helicity of peptide III(Glu-2→ Ala, Arg-10→ Ala, α-$CONH_2$→ α-COOH) that occurs as the α-COO^- group is protonated (Fig. 3) can be explained as arising from a charged-group:helix dipole interaction.

The Glu-$2^- \cdots$ Arg-10^+ Ion Pair

There is a well-defined H-bonded ion pair between the side chains of Glu-2^- and Arg-10^+ in the highly refined structure of RNase A from combined X-ray and neutron diffraction data (Wlodawer and Sjölin 1983). The interaction has been studied in experiments based on reconstitution of S-peptide and chemically synthesized analogs of S-peptide with S-protein (Hofmann et al. 1970; Marchiori et al. 1972). It is of major interest to determine whether or not the Glu-$2^- \cdots$ Arg-10^+ ion pair is present in the C-peptide helix, both because of its unusual nature (formed between residues that are spaced eight residues apart) and because it fixes the amino-terminal boundary of the helix between Glu-2 and Thr-3. It is sterically improbable to make this ion pair if Glu-2 is part of the helix, as can be shown with space-filling models.

One would like to analyze the Glu-$2^- \cdots$ Arg-10^+ interaction by the methods that have been used successfully to show that Glu-2^- and His-12^+ are responsible for the pH dependence of helicity: chemical modification or deletion of Glu-2 (Rico et al. 1984); replacement of Glu-2 or His-12 by Ala (Shoemaker et al. 1985); and synthesis of C-peptide analogs in which only Glu-2 or His-12 titrates in the pH range of interest (Shoemaker et al. 1987). There are, however, several problems with these approaches. First, Glu-2^- could stabilize the helix by interacting with the helix dipole (Shoemaker et al. 1985, 1987). Therefore, experiments showing that Glu-2^- is a helix-stabilizing residue do not establish that there is a Glu-$2^- \cdots$ Arg-10^+ ion pair. Second, the high pK of Arg-10 makes it difficult to employ pH titration.

The strategy used here is to study an analog of C-peptide with the replacement Arg-10→ Ala. If the Glu-$2^- \cdots$ Arg-10^+ ion pair exists and stabilizes the C-peptide helix, then the Arg-10→ Ala replacement should have two consequences. (1) The helix content at pH 5 should drop with Arg-10→ Ala. (2) The pH titration of Glu-2 without Arg-10 should no longer affect helicity by this mechanism, although Glu-2^- might instead stabilize the helix by interacting with the helix dipole. For illustration, consider first the effect of the replacement Glu-2→ Ala. Figure 5a shows the pH titration of the reference peptide (III), and Figure 5b shows the titration of III(Glu-2→ Ala). There is a large drop in helicity at pH 5 and the acid limb of the titration curve disappears with Glu-2→ Ala.

The effects of the replacement Arg-10→ Ala are shown in Figure 5c. There is no significant change in helicity at pH 5, but the acid limb of the titration curve again disappears. The latter effect shows that Glu-2^- and Arg-10^+ do interact, but does not establish whether the interaction is direct or indirect. The first effect suggests that, if there is a Glu-$2^- \cdots$ Arg-10^+ ion pair which contributes to helix stability, then breaking this interaction by Arg-10→ Ala still allows Glu-2^- to stabilize the helix by interacting with the helix dipole. If so, why doesn't pH titration of Glu-2^- to Glu-2^0 cause a drop in helicity (Fig. 5c)? Several explanations are possible, depending on the relative importance of the following effects as Glu-$2^- \rightarrow$ Glu^0: (1) loss of the helix-stabilizing interaction of Glu-2^- with the helix dipole; (2) intrinsic s value effects (Sueki et al. 1984); (3) a "helix-lengthening" reaction to include

Downloaded from symposium.cshlp.org on March 7, 2012 - Published by Cold Spring Harbor Laboratory Press

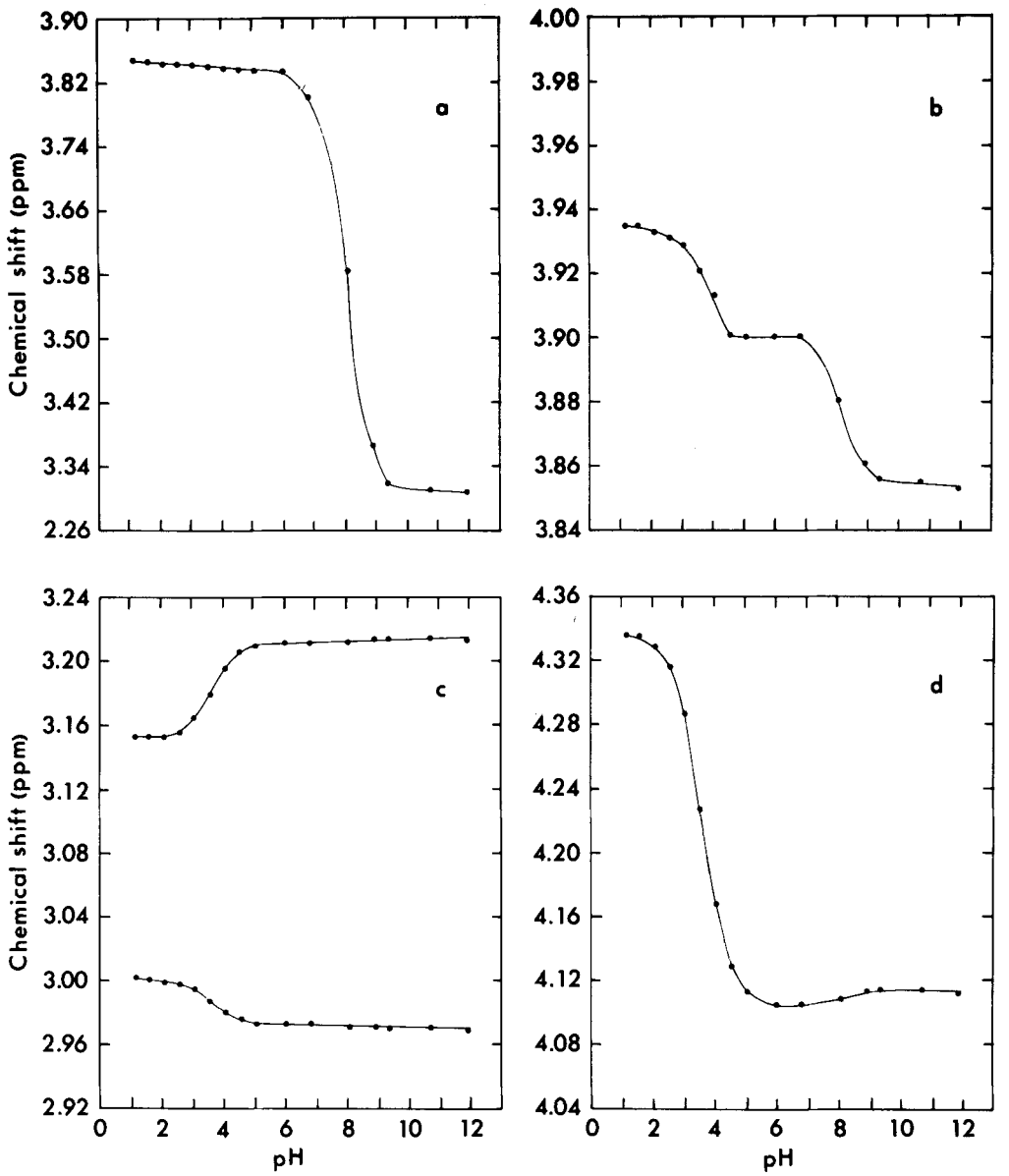

Figure 4. pH titration of the chemical shifts of some protons in the tetrapeptide Gly-Gly-Phe-Ala (0.01 M NaCl, 20°C, D_2O). The figure shows that substantial titration shifts are observed for some resonances (see *b* and *c*) of groups that are too far from the α-COOH group to be influenced by through-bond effects. Chemical shifts are reported relative to the internal standard sodium 3-trimethylsilyl-(2,2,3,3-$^2H_4$)propionate (TSP), and were corrected for the pH dependence of the TSP resonance. Measurements were made on a 500-MHz GE-Nicolet instrument. pH values in D_2O refer to uncorrected meter readings. There are likely to be negligible through-bond effects from the pH titration of the α-COOH group for protons beyond 5 bonds of the α-COOH group (Bundi and Wüthrich 1979). The number of bonds from the α-COO^- group to the αCH_2 of Gly-1 (*a*) is 11, to the αCH_2 of Gly-2 (*b*) is 8, to the $\beta\beta' CH_2$ resonances of Phe-3 (*c*) is 6, and to the αCH of Ala-4 (*d*) is 2. The titration shift for the αCH resonance of Ala-4 is upfield, as expected for a through-bond effect. In contrast, the shift for one of the Phe-8 $\beta\beta' CH_2$ resonances is downfield, indicative of a through-space interaction.

acetyl-Ala-1 and Glu-2. Experiments are in progress to address the role of each of these effects.

In summary, our results indicate that the Glu-$2^- \cdots$ Arg-10^+ ion pair is present in the C-peptide helix, but that our understanding of this interaction is still obscure, at least by the methods employed so far.

Aromatic Interaction between Phe-8 and His-12

The approach used here is to study peptide III with the replacement Phe-8→Ala. If an aromatic interaction between Phe-8 and His-12 makes an important contribution to helix stability, then the helicity might drop with Phe-8→Ala much as it drops with His-12→Ala (Fig. 6a). Figure 6b shows that there is a moderate drop in helicity with Phe-8→Ala. Also, NMR data (K.R. Shoemaker et al., in prep.) indicate that a Phe-8$\cdots$His-12 interaction can be detected.

If the aromatic interaction exists and is stronger with His-12^+ than with His^0 (A. Bierzynski, pers. comm.), then the alkaline limb might disappear with Phe-8→Ala, as it does with His-12→Ala (Fig. 6a). There

Downloaded from symposium.cshlp.org on March 7, 2012 - Published by Cold Spring Harbor Laboratory Press

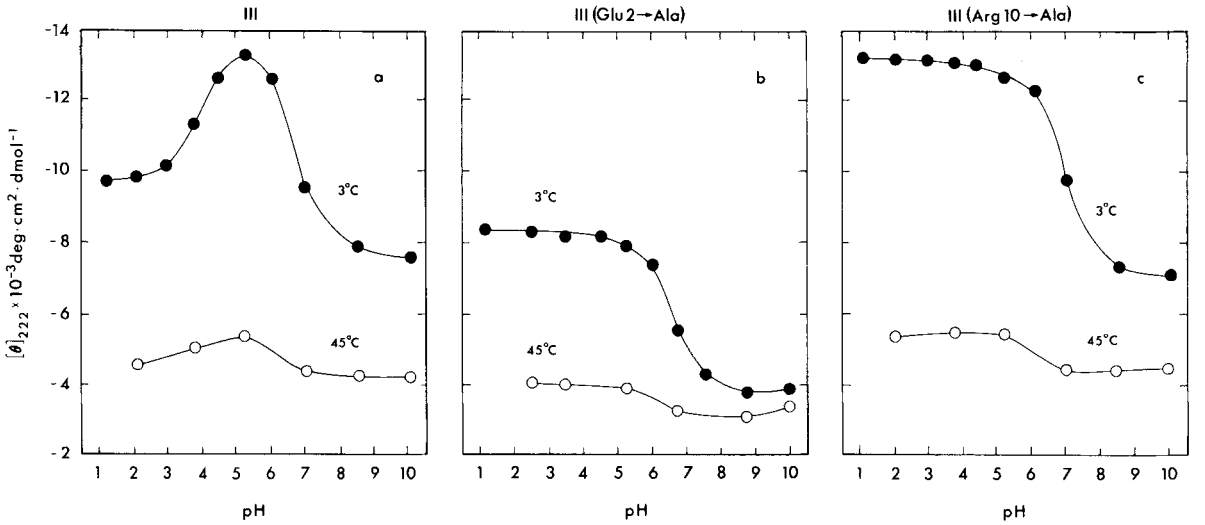

Figure 5. pH titration of helicity for three peptides used to study a possible Glu-2$^-$ · · · Arg-10$^+$ ion pair: reference peptide III (*a*) (Shoemaker et al. 1987), III(Glu-2→ Ala) (*b*), and III(Arg-10→ Ala) (*c*). The figure shows the effects on helix content of replacing Glu-2 or Arg-10 by Ala. Conditions: see Fig. 3. The sequence of III is acetyl-A-E-T-A-A-A-K-F-L-R-A-H-A-CONH$_2$.

are comparable decreases in the magnitudes of both the alkaline and acid limbs of the titration curve (Fig. 6b), showing that the change in the alkaline limb does not necessarily result from loss of a Phe-8 · · · His-12 interaction.

We conclude that a Phe-8 · · · His-12 aromatic interaction makes only a moderate contribution to C-peptide helicity and that it does not completely explain the role of His-12 in determining the pH dependence of C-peptide helix content. As with Glu-2$^-$, both helix dipole and intrinsic s value effects also need to be considered.

CONCLUDING REMARKS

A search for specific side-chain interactions that stabilize the C-peptide helix has thus far shown the following. (1) The interaction of charged groups near the ends of the helix with the α-helix macrodipole may either stabilize or destabilize the helix. For example, this interaction explains the destabilizing effects of charged α-NH$_3^+$ and α-COO$^-$ groups, and explains why Lys-1^{++} destabilizes the helix. (2) Glu-2$^-$ may control helicity by forming a Glu-2$^-$ · · · Arg-10$^+$ ion pair as it does in RNase A. This interaction is especially

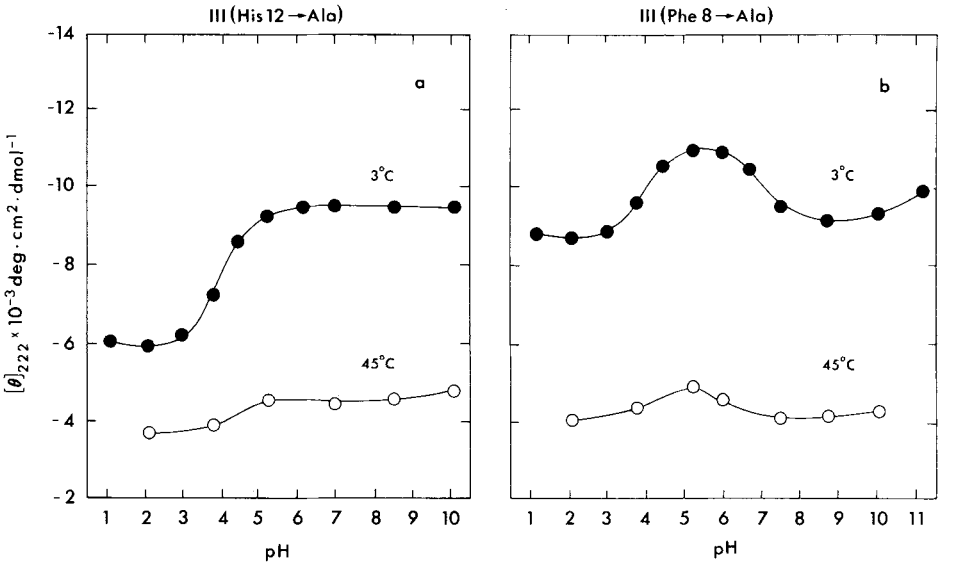

Figure 6. pH titration of helicity for two peptides used to study a possible aromatic interaction between Phe-8 and His-12: III(His-12→ Ala) (*a*) and III(Phe-8→ Ala) (*b*). See Fig. 5 for III. The figure shows the effects on helix content of replacing Phe-8 or His-12 by Ala. Conditions: see Fig. 3.

Downloaded from symposium.cshlp.org on March 7, 2012 - Published by Cold Spring Harbor Laboratory Press

important among the specific side-chain interactions because it fixes the amino-terminal boundary of the helix. (3) A helix-stabilizing role for His-12 is served by an aromatic interaction with Phe-8 and/or a helix dipole interaction. (4) It is known that there is also a "helix stop" in the S-peptide sequence that fixes the carboxy-terminal boundary of the helix (Rico et al. 1983; Kim and Baldwin 1984), but the nature of this stop signal remains to be determined. (5) Additional specific side-chain interactions that contribute to C-peptide helix content remain to be identified.

Although the magnitudes of these interactions are not large (each interaction stabilizes the helix only by a factor of 2 or 3), the interactions are important in two respects. First, they help to raise helicity into the range where it is measurable. For example, without the helix-stabilizing effects of Glu-2^- and His-12^+, the helicity of C-peptide is so low that the ability of C-peptide to form a helix in aqueous solution would not have been discovered (Fig. 2 of Bierzynski et al. 1982). Reference peptide III does show measurable helicity in the absence of Glu-2 (Fig. 5b) or His-12 (Fig. 6a), but only because III contains other helix-enhancing replacements. Second, although these interactions are small in magnitude, they are important in allowing the C-peptide helix to function as an autonomous folding unit. This effect is particularly striking in the case of the Glu-$2^- \cdots$ Arg-10^+ ion pair, which sets the amino-terminal boundary of the helix.

Because of competing interactions, determination of specific side-chain interactions in a helix formed by a peptide fragment of a protein is not simple. This point is made clear by the above discussion of the Glu-$2^- \cdots$ Arg-10^+ ion pair and the Phe-$8 \cdots$ His-12 interaction. For this reason, it is important that it is possible to analyze Glu$^- \cdots$ Lys$^+$ ion pairs in peptides of de novo design. These peptides have simple, repetitive sequences based on an alanine backbone (S. Marqusee and R.L. Baldwin, in prep.). This approach should simplify the problem of analyzing specific side-chain interactions in α helices.

ACKNOWLEDGMENTS

We thank Virginia MacCosham for technical assistance, and Jannette Carey, Homme Hellinga, Fred Hughson, Susan Marqusee, John Osterhout, and David Schultz for helpful discussion. This work was supported by National Institutes of Health grant GM-31475.

REFERENCES

Barden, J.A. and B.E. Kemp. 1987. NMR of a synthetic peptide spanning the triphosphate binding site of adenosine 5′-triphosphate in actin. *Biochemistry* **26:** 1471.

Bermejo, F.J., M. Rico, J. Santoro, J. Herranz, E. Gallego, and J.L. Nieto. 1986. Quantum-chemical calculations of a proposed Phe_n-His_{n+4} stabilizing interaction in peptide α-helices. *J. Mol. Struct.* **142:** 339.

Bierzynski, A., P.S. Kim, and R.L. Baldwin. 1982. A salt bridge stabilizes the helix formed by isolated C-peptide of RNase A. *Proc. Natl. Acad. Sci.* **79:** 2470.

Bierzynski, A., M. Dadlez, M. Sobociński, and G. Kupryszewski. 1986. Conformational study of two synthetic peptides with sequence analogies to the N-terminal fragment of RNase A. *Biophys. Chem.* **25:** 127.

Blackburn, P. and S. Moore. 1982. Pancreatic ribonuclease. In *The enzymes* (ed. P.D. Boyer), vol. 15, p. 317. Academic Press, New York.

Blagdon, D.E. and M. Goodman. 1975. Mechanisms of protein and polypeptide helix initiation. *Biopolymers* **14:** 241.

Blundell, T., J. Singh, J. Thornton, S.K. Burley, and G.A. Petsko. 1986. Aromatic interactions. *Science* **234:** 1005.

Brown, J.E. and W.A. Klee. 1971. Helix-coil transition of the isolated amino terminus of ribonuclease. *Biochemistry* **10:** 470.

Bruist, M.F., S.J. Horvath, L.E. Hood, T.A. Steitz, and M.I. Simon. 1987. Synthesis of a site-specific DNA-binding peptide. *Science* **235:** 777.

Bundi, A. and K. Wüthrich. 1979. Use of amide ^{1}H-NMR titration shifts for studies of polypeptide conformation. *Biopolymers* **18:** 299.

Burgess, A.W., P.K. Ponnuswamy, and H.A. Scheraga. 1974. Analysis of conformations of amino acid residues and prediction of backbone topography in proteins. *Isr. J. Chem.* **12:** 239.

Burley, S.K. and G.A. Petsko. 1985. Aromatic-aromatic interaction: A mechanism of protein structure stabilization. *Science* **229:** 23.

Chou, P.Y. and G.D. Fasman. 1974. Conformational parameters for amino acids in helical, β-sheet, and random coil regions calculated from proteins. *Biochemistry* **13:** 211.

———. 1978. Prediction of the secondary structure of proteins from their amino acid sequence. *Adv. Enzymol.* **47:** 45.

Craik, C.S., S.R. Buchman, and S. Beychok. 1981. O_2 binding properties of the product of the central exon of β-globin gene. *Nature* **291:** 87.

Doolittle, R.F. 1985. The genealogy of some recently evolved vertebrate proteins. *Trends Biochem. Sci.* **10:** 233.

Dyson, H.J., K.J. Cross, R.A. Houghten, I.A. Wilson, P.E. Wright, and R.A. Lerner. 1985. The immunodominant site of a synthetic immunogen has a conformational preference in water for a type-II reverse turn. *Nature* **318:** 480.

Feeney, J. 1976. Improved component vicinal coupling constants for calculating side-chain conformations in amino acids. *J. Magnet. Res.* **21:** 473.

Gilbert, W. 1978. Why genes in pieces? *Nature* **271:** 501.

———. 1985. Genes-in-pieces revisited. *Science* **228:** 823.

Gō, M. 1981. Correlation of DNA exonic regions with protein structural units in haemoglobin. *Nature* **291:** 90.

Goren, H.J., M. Fridkin, E. Katchalski-Katzir, and N. Lotan. 1979. Conformational changes of poly(L-histidyl-L-alanyl-α-L-glutamic acid) in solution. Transition pathways and conformational intermediates. *Biopolymers* **18:** 981.

Hofmann, K., J.P. Visser, and F.M. Finn. 1970. Studies on polypeptides. XLIV. Potent synthetic S-peptide antagonists. *J. Am. Chem. Soc.* **92:** 2900.

Hol, W.G.J. 1985. The role of the α-helix dipole in protein function and structure. *Prog. Biophys. Mol. Biol.* **45:** 149.

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

Kabsch, W. and C. Sander. 1984. On the use of sequence homologies to predict protein structure: Identical pentapeptides can have completely different conformations. *Proc. Natl. Acad. Sci.* **81:** 1075.

Kim, P.S. and R.L. Baldwin. 1984. A helix stop signal in the isolated S-peptide of ribonuclease A. *Nature* **307:** 329.

Kim, P.S., A. Bierzynski, and R.L. Baldwin. 1982. A competing salt bridge suppresses helix formation by the isolated C-peptide carboxylate of ribonuclease A. *J. Mol. Biol.* **162:** 187.

Knight, K.L. and K. McEntee. 1986. Nucleotide binding by a 24-residue peptide from the RecA protein of *Escherichia coli*. *Proc. Natl. Acad. Sci.* **83:** 9289.

Downloaded from symposium.cshlp.org on March 7, 2012 - Published by Cold Spring Harbor Laboratory Press

Kopple, K.D., G.R. Wiley, and R. Tauke. 1973. A dihedral angle-vicinal proton coupling constant correlation for the α-β bond of amino acid residues. *Biopolymers* **12:** 627.

Lenstra, J.A., J. Hofsteenge, and J.J. Beintema. 1977. Invariant features of the structure of pancreatic ribonuclease. A test of different predictive models. *J. Mol. Biol.* **109:** 185.

Lim, V.I. 1974. Algorithms for prediction of α-helical and β-structural regions in globular proteins. *J. Mol. Biol.* **88:** 873.

Marchiori, F., G. Borin, L. Moroder, R. Rocchi, and E. Scoffone. 1972. Relation between structure and function in some partially synthetic ribonucleases S′. I. Kinetic determinations. *Biochim. Biophys. Acta* **257:** 210.

Maxfield, F.R. and H.A. Scheraga. 1975. The effect of neighboring charges on the helix forming ability of charged amino acids in proteins. *Macromolecules* **8:** 491.

Mitchinson, C. and R.L. Baldwin. 1986. The design and production of semisynthetic ribonucleases with increased thermostability by incorporation of S-peptide analogues with enhanced helical stability. *Proteins: Struct. Funct. Genet.* **1:** 23.

Pachler, K.G.R. 1964. Nuclear magnetic resonance study of some α-amino acids. II. Rotational isomerism. *Spectrochim. Acta* **20:** 581.

Perutz, M.F., J.C. Kendrew, and H.C. Watson. 1965. Structure and function of haemoglobin. II. Some relations between polypeptide chain configuration and amino acid sequence. *J. Mol. Biol.* **13:** 669.

Ramachandran, J., A. Berger, and E. Katchalski. 1971. Synthesis and physicochemical properties in aqueous solution of the sequential polypeptide poly(tyr-ala-glu). *Biopolymers* **10:** 1829.

Rico, M., E. Gallego, J. Santoro, F.J. Bermejo, J.L. Nieto, and J. Herranz. 1984. On the fundamental role of the Glu-$2^{-}\cdots$Arg-10^{+} salt bridge in the folding of isolated ribonuclease A S-peptide. *Biochem. Biophys. Res. Commun.* **123:** 757.

Rico, M., J.L. Nieto, J. Santoro, F.J. Bermejo, J. Herranz, and E. Gallego. 1983. Low-temperature ^{1}H-NMR evidence of the folding of isolated ribonuclease S-peptide. *FEBS Lett.* **162:** 314.

Rico, M., J. Santoro, F.J. Bermejo, J. Herranz, J.L. Nieto, E. Gallego, and M.A. Jiménez. 1986. Thermodynamic parameters for the helix-coil thermal transition of ribonuclease S-peptide and derivatives from ^{1}H-NMR data. *Biopolymers* **25:** 1031.

Rose, G.D. 1979. Hierarchic organization of domains in globular proteins. *J. Mol. Biol.* **134:** 447.

Schechter, B., I. Schechter, J. Ramachandran, A. Conway-Jacobs, and M. Sela. 1971. The synthesis and circular dichroism of a series of peptides possessing the structure $(\text{L-tyrosyl-L-alanyl-L-glutamyl})_n$. *Eur. J. Biochem.* **20:** 301.

Sheridan, R.P., R.M. Levy, and F.R. Salemme. 1982. α-helix dipole model and electrostatic stabilization of 4-α-helical proteins. *Proc. Natl. Acad. Sci.* **79:** 4545.

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

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

Sueki, M., S. Lee, S.P. Powers, J.B. Denton, Y. Konishi, and H.A. Scheraga. 1984. Helix-coil stability constants for the naturally occurring amino acids in water. 22. Histidine parameters from random poly[(hydroxybutyl)glutamine-*co*-L-histidine]. *Macromolecules* **17:** 148.

Sundaralingam, M., W. Drendel, and M. Greaser. 1985. Stabilization of the long central helix of troponin C by intrahelical salt bridges between charged amino acid sidechains. *Proc. Natl. Acad. Sci.* **82:** 7944.

Wada, A. 1976. The α-helix as an electric macrodipole. *Adv. Biophys.* **9:** 1.

Wilson, P.T., T.L. Lentz, and E. Hawrot. 1985. Determination of the primary amino acid sequence specifying the α-bungarotoxin binding site on the α subunit of the acetylcholine receptor from *Torpedo californica*. *Proc. Natl. Acad. Sci.* **82:** 8790.

Wlodawer, A. and L. Sjölin. 1983. Structure of ribonuclease A: Results of joint neutron and X-ray refinement at 2.0-Å resolution. *Biochemistry* **22:** 2720.

View publication stats