PROTEINS: Structure, Function, and Genetics 1:211–217 (1986)

Stabilization of the Ribonuclease S-Peptide α-Helix by Trifluoroethanol

Jeffrey W. Nelson and Neville R. Kallenbach
Department of Biology, University of Pennsylvania, Philadelphia, Pennsylvania 19104

ABSTRACT **The effects of trifluoroethanol (TFE) on the stability of the α-helix formed by ribonuclease S-peptide, residues 1–19 of ribonuclease A, were studied by measuring circular dichroism as a function of TFE concentration, pH, and temperature. The S-peptide forms an unusually stable α-helix, which is known to be stabilized by TFE. The magnitude of the effect of charged groups on the peptide, manifested by the change in α-helix stability as a function of pH, was not altered significantly by either TFE concentration or temperature, indicating that the lower dielectric constant of TFE is not important in the stabilization of this α-helix. This suggests that the α-helix might be stabilized by many interactions in addition to the effects of charges. The titration curve of circular dichroism vs. TFE concentration appears to be cooperative at 0°C, but becomes progressively less cooperative at temperatures between 25 and 75°C. The properties of the TFE stabilization indicate that TFE might be a useful probe with which to measure the stability of marginally stable peptides and small proteins.**

Key words: protein folding, α-helix stabilization, peptide structural stability, circular dichroism, protein electrostatics

INTRODUCTION

The structural stability of marginally stable small peptides and the physical interactions accounting for the stability are of current interest in order to understand the biological actions of peptides as well as the mechanism of early steps of protein folding. The problem is complicated by the fact that very few linear peptides have stable structures. In protein folding, the stability of intermediates in the folding reaction is generally marginal, although this stability might nevertheless guide the folding pathway. We are attempting to develop a general scheme to assess quantitatively the stabilities of marginally stable peptides and proteins by using structure-forming solvents such as trifluoroethanol (TFE). The idea is to measure the extent of structure of a peptide by using CD, for example, as increasing amounts of stabilizer are added. The stability of the structure might be estimated by the concentration of stabilizer necessary to induce structure.

For a model system, we have chosen the S-peptide (residues 1–19 of ribonuclease A), which is unusual in forming a unimolecular α-helical structure between residues 3 and 13[1]; this structure is also present in the parent protein. The sequence of the S-peptide (1–19) is shown below. The C-peptide, which is composed of residues 1–13, has also been studied extensively; based on circular dichroism measurements, it has been estimated to have approximately 30% α-helix at 0°C and at the optimal pH in aqueous solution.[2] The stability of both the S-peptide and C-peptide depends on the pH of the solution, an effect that has been attributed to the charges of the titratable side groups. The C-peptide α-helix exhibits maximum stability at pH 5.0,[3] whereas in the S-peptide helix is most stable at pH 3.8.[4] Both peptides show reduced helix content at pH values above and below the optimal pH.

Scheme 1: Sequence of S-peptide (1–19)
lys-glu-thr-ala-ala-ala-lys-phe-glu-arg-gln-his-met-asp-ser-ser-thr-ser-ala

Here we describe a circular dichroism (CD) investigation of the effects of trifluoroethanol on the stability of the S-peptide directed at elucidating the mechanism of stabilization insofar as this is possible. Our objective is to determine if TFE has properties which make it useful as a general probe for determining peptide structural stabilities. In particular, we would like to address several questions about the TFE stabilization: Does TFE amplify the charged-group effect; i.e., do charges become more important due to a decrease in the bulk solvent dielectric constant? Does TFE preferentially stabilize subdomains of the structure or is the whole sequence equally stabilized? Does TFE affect the cooperativity of the transition; in other words, how does TFE affect helix initiation and propagation? And finally, does TFE have properties that render it useful as a general probe to determine stabilities of marginally stable peptide structures?

MATERIALS AND METHODS

The S-peptide, residues 1–19 of ribonuclease A, was kindly provided by Dr. Robert L. Baldwin. The concentration of a stock solution of S-peptide in H_2O was

Received July 21, 1986; accepted August 15, 1986.

J.W. Nelson's present address is Department of Biochemistry, Louisiana State University, Baton Rouge, LA 70803. Address reprint requests to him there.

determined by hydrolyzing the peptide by autoclaving in 13 M NaOH, followed by ninhydrin analysis (Dr. Peter S. Kim, personal communication). [5] A solution of leucine, treated identically, was used as the concentration standard. The concentration determination was accurate to about 10%. All solutions for study were prepared by making dilutions of this stock solution by weight by using the density of each solvent. The dilutions of the stock solution were on the order of 1:150 and thus did not significantly alter the composition of the solvent. Relative magnitudes of the molar properties are more accurate than the absolute values.

The 2,2,2-trifluoroethanol (TFE) was the Gold Label grade from Aldrich Chemical Company. The other chemicals were of reagent grade and all were used without further purification. All water was deionized and quartz-distilled.

pH Measurements

The pH* measurement of mixtures of TFE and water requires the definition of a pH standard state for each solvent composition. pH* designates that the solvent was a mixture of water and TFE. We used a procedure in which a solution of 0.01 N HCl is assigned a pH* value by assuming that it fully dissociates and by using the Debye-Hückel theory to calculate the activity coefficients.[6] Each solution of HCl in the mixture of water and TFE was thus assigned a pH* of 2.07. After calibrating the pH meter and electrode between pH 2.00 and 10.00 in aqueous solution, the pH meter was set to 2.07 on the TFE-water-HCL solution, and the electrode was assumed to respond linearly with pH* changes. For routine work, the pH*s of buffers consisting of 0.01 M acetic acid and 0.01 M sodium acetate were determined by the above procedure, and these buffers were used for subsequent recalibration of the pH meter. The pH* values of these buffers for different TFE:water mixtures are given in Table I. The pH* measurements are probably accurate to about 0.05 pH* units.

Circular Dichroism Measurements

The circular dichroism (CD) measurements were made on a modified Cary 60 circular dichroism spectrophotometer (Aviv Associates model 60DS) interfaced to a microcomputer. The temperature was controlled by a Hewlett Packard model 89100A temperature control accessory interfaced to the computer in the CD. Temperature settings were monitored at the cell jacket and were found to be within 0.2°C of the temperature inside the cuvette. The ellipticity was calibrated within 1% by using (+) -10 camphorsulfonic acid (Sigma) and by assuming a molar extinction coefficient at 285 nm of 34.5 $M^{-1} \cdot cm^{-1}$ and a molar ellipticity at 290.5 nm of $7{,}780° \cdot cm^2 \cdot dmole^{-1}$,[7] and the wavelength was calibrated to within 0.3 nm by using the 266.7-, 260.0-, 258.8-, and 252.9-nm lines of benzene vapor.

TABLE I. pH* Values for 0.01 M Acetic Acid and 0.01 M Sodium Acetate in Trifluoroethanol (TFE): Water Mixtures

Mole % TFE	pH*
0	4.68
10	5.31
20	5.77
40	6.64

Cuvettes of either 1- or 10-mm pathlength were used and were either conventional stoppered rectangular cuvettes (Hellma) or were custom-made cylindrical cuvettes with 13 mm outside diameter (Hellma). All cuvettes exhibited moderate CD baselines, normally less than 3 millidegrees, and were measured at each temperature. Ellipticities are reported as the mean residue ellipticity, which is the ellipticity per mole of peptide strand divided by 19.

CD Spectra of the S-Peptide in 10 Mole % TFE

CD spectra from 270 to 185 nm were obtained for a solution of the S-peptide in a buffer consisting of 0.1M $NaClO_4$ in 10 mole % TFE, with the pH* adjusted to 2.07 with $HClO_4$ in the same solvent. $NaClO_4$ was used because of its transparency in the far UV. The pathlength was 1 mm. Single scans were made at temperatures between 0 and 75°C; the wavelength was stepped by 0.2 nm; a 1.5-nm bandwidth was used; and averaging was done at each wavelength for 2 seconds. The data were smoothed by replacing each point by the average of five points (itself and two on either side) and were baseline corrected. Spectra at 25°C before and after the experiment were superimposable within the noise.

pH* Titrations of S-Peptide Stability

The effect of pH* on the α-helix stability was determined by measuring the ellipticity at 222 nm of solutions of approximately 25 μM S-peptide strands in 0.1 M $NaClO_4$ at TFE concentrations of 0, 10, 20, and 40 mole % in a 10-mm pathlength cuvette. The pH* of the solution in the cuvette was measured; CD measurements were made at 0, 25 and 50°C; and the pH* was remeasured and changed by adding to the cuvette either $HClO_4$ or NaOH solutions in the same buffer. The pH*s before and after each measurement were generally within 0.13 pH* units. The experiment started at a pH* near the middle of the range, first increasing stepwise to about pH*9 and then decreasing to about pH*2. The measurement was repeated at the starting pH*, and the two measurements agreed to about 3%. The peptide concentration was always corrected for the small amount of acid or base solution added; the concentration was reduced about 3% by the end.

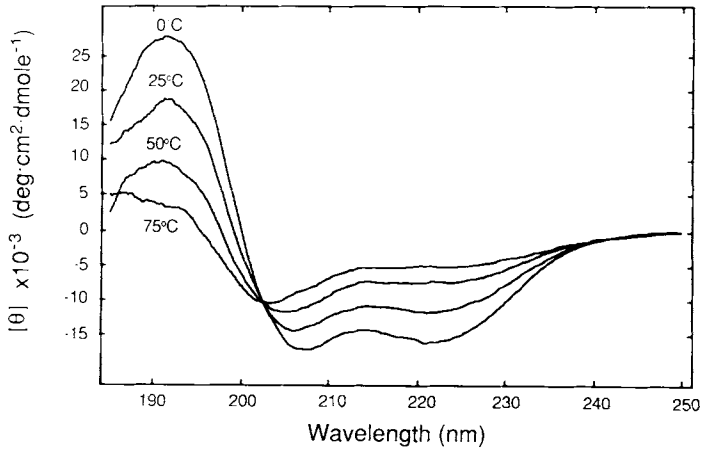

Fig. 1. Circular dichroism spectra of the mean residue ellipticity of the S-peptide (1–19) in 10 mole % trifluoroethanol (TFE), 0.1 M $NaClO_4$, and pH* = 2.07 ($HClO_4$). Spectra were taken at 0, 25, 50, and 75°C and were smoothed and baseline-corrected as described in the text. The concentration of the S-peptide was 51 μM in peptide strand (0.97 mM in amino acids) with a pathlength of 1 mm.

TFE Titrations of S-Peptide

The effect of TFE concentration on the S-peptide α-helix stability was determined by measuring the ellipticity at 222 nm for solutions with increasing amounts of TFE. To facilitate comparisons with earlier studies, we used a solvent consisting of 0.1 M NaCl, 0.01 M HCl, and 0–70 mole % TFE. The helix is maximally stable at this pH* (2.07) in TFE (see Fig. 2). $NaClO_4$ and $HClO_4$ had to be used instead of NaCl and HCl at TFE concentrations of 50 mole % or higher, due to insolubility of NaCl. Concentrations of the peptide strands were approximately 25 μM. Measurements were made in 10-mm pathlength cuvettes at 0, 25, 50, and 75°C.

Testing for Aggregation

The possibility of aggregation of the S-peptide in TFE solutions was tested by measuring the molar ellipticity of the S-peptide over a range of concentrations, with 0.1 M $NaClO_4$ in 40 mole % TFE as the solvent. Between S-peptide strand concentrations of 0.024 to 0.27 mM the molar ellipticity at 222 nm changed less than 5%, indicating no significant degree of aggregation at these concentrations.[8]

RESULTS

Figure 1 shows the CD spectra for the S-peptide (1–19) in 10 mole % TFE, 0.1 M $NaClO_4$, at pH* = 2.07. Spectra were taken at 0, 25, 50, and 75°C. By comparing the shapes of the curves, it is clear that as the temperature is increased, the structural transition from α-helix to random-coil is taking place. The very clean isodichroic point at 202 nm suggests that the transition is two-state, so that each amino acid is either in a helical state or a random coil state. The minima in ellipticity at 208 and 222 nm are diagnostic for α-helices. The ellipticity at 222 nm was monitored in subsequent experiments as an indication of the extent of helix formation.

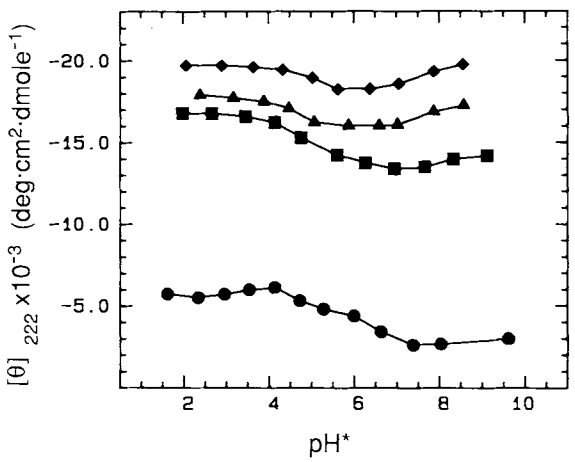

Fig. 2 The pH* titration curve of the mean residue ellipticity at 222 nm of S-peptide in different TFE concentrations at 0°C: No TFE (●), 10 mole % TFE (■), 20 mole % TFE (▲), and 40 mole % TFE (♦). The solvents contained 0.1 M $NaClO_4$. The experimental procedures are described in the text. The concentrations of the peptide (in peptide strand) were 25 to 26 μM with a pathlength of 10 mm.

Figure 2 shows the pH* titration curves of the S-peptide in solutions of 0, 10, 20, and 40 mole % TFE and 0.1 M $NaClO_4$ at 0°C. The shape of the curve in the absence of TFE, where the stability exhibits a maximum near pH 4, is due to changes in the charges of the titratable side groups as the pH is altered. This dependence on pH has been referred to as the "charged-group effect".[3] The curves at higher TFE concentrations have a similar shape, except that they are displaced progressively to higher α-helix content (as indicated as a more negative value of $[\theta]_{222}$), and the slight destabilization as the pH* is lowered below 4 is not observed in TFE solutions. Thus, the magnitude of the charged-group effect does not appear to change significantly at higher TFE concentrations.

The effect of temperature on the pH* titration curve of S-peptide in 10 mole % TFE and 0.1 M $NaClO_4$ is

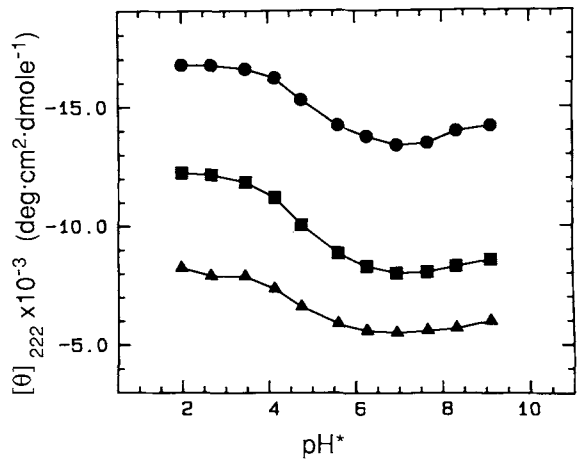

Fig. 3. The pH* titration curve of the mean residue ellipticity at 222 nm of S-peptide in 10 mole % TFE, 0.1 M $NaClO_4$, at 0°C (●), 25°C (■), and 50°C (▲). The experimental procedures are described in the text. The concentrations of the peptide (in peptide strand) were 25 to 26 μM with a pathlength of 10 mm.

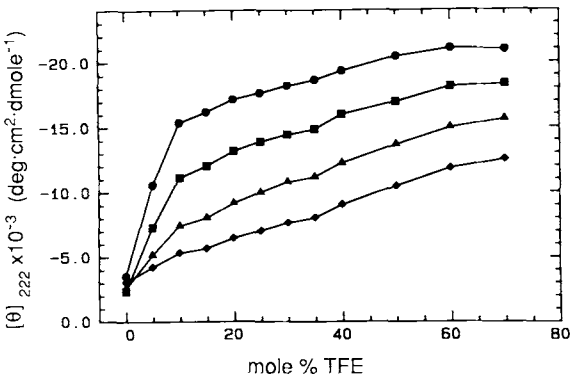

Fig. 4. The curve of the mean residue ellipticity vs. solvent TFE concentration, at 0°C (●), 25°C (■), 50°C (▲), and 75°C (♦). Solutions below 50 mole % TFE contained 0.1 M NaCl and 0.01 N HCl, whereas at or above 50 mole % TFE, insolutility of NaCl necessitated the use of 0.1 M $NaClO_4$ and 0.01 M $HClO_4$ instead. The concentrations of the peptide (in peptide strand) were 25 to 26 μM with a pathlength of 10 mm.

shown in Figure 3. The stability of the α-helix is reduced at higher temperatures, but the magnitude of the charged-group effect is not substantially affected over the range of 0 to 50°C, except for a slight decrease in the change in ellipticity vs. pH* at 50°C relative to the lower temperatures.

The TFE titration curve of α-helix formation is shown in Figure 4. The mean residue ellipticity is plotted vs. the mole % TFE concentration, at 0, 25, 50, and 75°C in a solution containing 0.1 M NaCl and 0.01 N HCl. This is the pH* (2.07) of maximal stability in TFE solutions (Fig. 2, 3). Using NaCl instead of $NaClO_4$ facilitates comparisons with earlier studies. However, because of the insolubility of NaCl, 0.1 M $NaClO_4$ and 0.01 N $HClO_4$ were used instead of NaCl and HCl at TFE concentrations of 50 mole % or above. At 0°C, the titration curve appears cooperative, since the α-helix content increases significantly as the concentration of TFE is increased over the range from 0 to 10 mole %.

Comparing the curves, it appears, first, that the apparent cooperativity with respect to TFE concentration decreases at higher temperatures, as shown by the dramatic decrease in the slope of the curves at low TFE concentrations. Second, the transitions at TFE concentrations above 10 mole % are not cooperative with respect to temperature, since the ellipticity increases roughly linearly with temperature over the range of 0 to 75°C, resulting in nearly parallel curves.

DISCUSSION

According to the Zimm-Bragg model, [9] the stability of an α-helix can be described by two parameters. The helix initiation parameter, σ, describes the effective nucleation of the α-helix, which requires the ordering of four successive amino acids to form the first hydrogen bond, while the helix propagation parameter, s, describes extension of an existing α-helix upon ordering the next amino acid, forming a hydrogen bond. Experimental values determined by using the host-guest procedure [10] suggest that the cooperativity parameter, σ, is very small, and the helix propagation factor, s, is near unity for all amino acids, with relatively little dependence on temperature. This indicates a cooperative transition, and would predict that the C- and S-peptides should not form stable helices. Baldwin and his colleagues have calculated that the measured stability of the C-peptide would require an average s-parameter of around 1.7, nearly twice the values determined by Scherage.[3] The question, then, is whether or not the Zimm-Bragg theory adequately describes the process of α-helix formation in real proteins and peptides.

Studies on the C-peptide have been most illuminating on the important factors in the stabilization of the α-helix. In even this most desirable case, the stability is still marginal, with a maximum of approximately 30% α-helix in aqueous solution.[2] This system shows the importance of the charged-group effect on helix stability, since the stability changes markedly as the pH of the solution is altered, due to changing the charges of the titratable side chains. From this study, the groups glu-2 and his-12 were identified by substitution experiments as the groups responsible.[3] The charge at the end of the helix has also been found to be important.[11] The charged-group effect is probably a result of several factors, including salt bridges between side chains[2,12] and the effects of charged groups on compensating the electric macrodipole induced by aligning the polar amide groups along the helix.[3]

It is important to develop a method whereby the stability of marginally stable peptides can be determined. We have proposed the use of trifluoroethanol (TFE), which is well known to stabilize α-helix structure in peptides. The idea is to monitor the structure of the peptide as the TFE concentration is increased, precisely the opposite to a urea denaturation curve of

a globular protein. To do this, we need an understanding of the effects of TFE, with the goal of determining its suitability in such studies. No detailed study has been made on the influence of charged groups of a peptide on the stabilization by TFE.

The S-peptide provides an ideal model system with which to probe the effects of TFE. The S-peptide forms a marginally stable α-helix in aqueous solution, exhibits a pronounced charged-group effect,[3] and normally forms a helix over only a portion of its sequence, from residues 3 to 13.[1] Previous studies have taken advantage of the stabilization of the S-peptide by TFE to determine the effects of amino acid substitutions on the α-helix stability.[13,14] Very recently an attempt has been made to estimate the stability of the S-peptide α-helix in both aqueous and TFE-containing solvents by using nuclear magnetic resonance (NMR) chemical shifts, assuming an all-or-none transition.[15]

The Physics of TFE

The dielectric constant of TFE is about one-third that of water, 26.67 vs. 78.54, respectively, at 25°C.[16] Interactions between charged species would be expected to be stronger in TFE solutions. Also compared to water, TFE is a much weaker base ($pKa_1 \sim -8.2$ for TFE vs. ~ -1.8 for water) and a stronger acid (pKa_2 = 12.4 vs. 15.3).[16] This means that TFE is stronger at donating protons for hydrogen bonds but weaker at accepting protons in hydrogen bonds. In mixed cosolvents of water and TFE, the properties will be intermediate between the two.

The effects of solvent acidity and basicity on the peptide amides have been probed by NMR.[16] Hydrogen bonding from the peptide amide proton (NH) to a solvent acceptor results in decreased electronic shielding of the proton, causing a shift of the ^1H NMR resonance to lower field. Hydrogen bonding from a solvent donor to the amide carbonyl (C=O) also deshields the proton due to indirect electronic effects. In a peptide amide with the NH exposed to solvent but the C=O buried, the chemical shift of the amide proton moves to higher field upon changing the solvent from H_2O to TFE. This is a result of the increased shielding due to reduced hydrogen bonding from the NH to the less basic TFE. When the C=O is exposed, but the NH is buried, the chemical shift moves to lower field, a result of decreased shielding due to increased hydrogen bonding of the more acidic TFE proton to the C=O. When both NH and C=O are exposed, the effect of the basicity on the NH predominates.

These properties suggest some possible interactions which might be responsible for stabilization of the S-peptide α-helix by TFE. As the TFE concentration increases, charged groups might be expected to become more important due to a lowering of the dielectric constant. The relative stabilities of the hydrogen bonds from the amide proton to the carbonyl in the α-helix and the solvent might also change, which would affect the extent of stabilization due to α-helix hydrogen bonds. Additional effects might be dehydration due to the lowering of water chemical potential, binding of TFE to the peptide, and changes in the water structure.

The Nature of the Transition Induced by TFE

The shapes of the CD spectra in Figure 1 indicate that the transition induced by TFE is from random coil to an α-helix, as expected. The isodichroic point at 202 nm is consistent with the idea that each amino acid is involved in a two-state transition, meaning that a residue is either in an α-helical or random coil state. This does not imply that the formation of the entire α-helix is two-state, or that the entire peptide is either completely helical or random coil. The extent of cooperativity of the α-helix formation must be determined from other experiments.

TFE and the Charged Group Effect

The most striking observation in these experiments is that, to a first approximation, TFE does not significantly alter the magnitude of the stabilization contributed by the charged groups. By comparing the curves at different concentrations of TFE at a constant temperature, it can be seen that the amount that the ellipticity changes at different pH* values is roughly the same in both aqueous solution and at all TFE concentrations, except that the slight destabilization at pH* values below the optimum is not observed in TFE solutions. The data at 0°C are shown in Figure 2; the same conclusion is reached from data at the other temperatures (data not shown). In addition, examining data at a fixed TFE concentration, while varying the temperature, also indicates the same conclusion (Fig. 3). This suggests that the stabilization by TFE does not involve magnification of the interactions between charged groups, since this would imply a much greater charged-group effect as the TFE concentration increases.

From earlier studies on the C- and S-peptides, the charged-group effect was found to be the predominant effect, since the helix was moderately stable at the optimal pH and much less stable at other pHs. We observe that increasing the concentration of TFE appears to shift the pH* curve of the S-peptide to higher stabilities, although the shapes of the pH* titration curves remain very similar. This suggests that the free energy contributed by charged groups is independent of temperature and TFE concentration. For example, we can write $\Delta G_{helix} = \Delta G_{charge} + \Delta G'$, where ΔG_{helix} is the total free energy of α-helix formation, ΔG_{charge} is the amount of free energy contributed by the charged groups, and $\Delta G'$ includes all other contributions. By definition, ΔG_{charge} depends on the charges of the residues, and thus pH, whereas $\Delta G'$ is independent of charges. We can set $\Delta G_{charge} = 0$ at a nonoptimal alkaline pH. Our results suggest that ΔG_{charge} is independent of both temperature and TFE concentration, whereas $\Delta G'$ depends on both temperature and TFE concentration. In aqueous solutions at

the optimal pH, $\Delta G'$ is slightly greater than 0, ΔG_{charge} is slightly less than 0, with the result that $\Delta G_{helix} \simeq 0$, whereas at a nonoptimal pH, where $\Delta G_{charge} = 0$, $\Delta G_{helix} = \Delta G'$ is slightly greater than 0, and little helix forms. In TFE, it appears that $\Delta G' < \Delta G_{charge}$, and ΔG_{charge} only slightly affects the extent of helix formation.

The observation that TFE affects interactions other than the charged-group effect might indicate that many interactions are important for α-helix stability. The S-peptide α-helix is only marginally stable in aqueous solution, and thus the stabilizing forces are probably small in magnitude. A shift of fractions of a kcal in the free energy can make noticeable changes in the α-helix stability. Even the charged-group effect is complex, since charged groups both interact to make salt bridges as well as compensate the helix dipole moment.[2,3,11]

TFE and Cooperativity

CD measurements can only determine the average content of α-helix, and can say little directly about the cooperativity of the α-helix transition. There are two types of cooperativity involved here: first the cooperativity of the α-helix transition, as manifested as an all-or-none behavior in the formation of α-helices, and then the cooperativity of helix content vs. TFE concentration—namely, whether the transition from random coil to α-helix occurs over a narrow range of TFE concentrations. These can be described in terms of the Zimm-Bragg σ and s parameters, which describe the transition, and in terms of the effects of TFE and temperature upon them.

We can address the second type of cooperativity by examining Figure 4, which shows the ellipticity of the S-peptide at different TFE concentrations and temperatures. At 0°C, the curve appears cooperative, in that the α-helix content increases greatly as the TFE concentration is increased. At higher temperatures, the transition looks much less cooperative. Thus, the temperature seems to change the basic shapes of the curves instead of just shifting the titration curve to higher or lower TFE concentrations. We cannot rationalize this in terms of the effects of TFE on σ and s parameters without more specific information. From a practical standpoint of determining relative stabilities, we want to perform the TFE studies at low temperatures, where the transition is more cooperative and we can see the differential stabilities more clearly.

In order to obtain a more detailed picture of the effects of TFE on the cooperativity of the α-helix to random coil transition, we need a way to measure the extent of helical structure at each amino acid. We are in the process of using NMR to probe each amino acid in order to address this question more directly.

TFE and the Helix Stop Signal

As mentioned before, the S-peptide has what is termed a "helix stop signal," which terminates the helix after residue 13.[1] The existence of this stop signal allows us to address the question of whether TFE stabilizes all amino acids equally or whether the stabilization is selective. We can calculate approximately what ellipticity we would expect if TFE stabilized only the 3–13 helix. A mean residue ellipticity at 222 nm of $-38{,}000° \cdot cm^2 \cdot dmole^{-1}$ was measured at 20°C for a tetradecapeptide of L-Met in TFE, which is α-helical,[17] whereas a value of $-38{,}500° \cdot cm^2 \cdot dmole^{-1}$ was measured for poly (γ-ethyl-L-glutamate) in TFE at 3°C.[14] Using a mean residue ellipticity of +3,900 for the random coil,[18] and −38,000 for the α-helix, we can calculate an expected ellipticity of approximately −19,000 if the helix is formed only from residues 3–13 (10 of 18 amide bonds are in an α-helix). From Figure 4, we see that at 0°C, the ellipticity levels off at a value near −20,000, which might suggest that the helix does not form over the entire length. Of course, this is indirect evidence because of the uncertainty of the values of the α-helix and random coil ellipticities and because we do not know in detail the cooperativity of the equilibrium under these conditions.

CONCLUSIONS

We can conclude several things about the effects of TFE on the α-helix formation in the S-peptide (1–19). TFE does not alter significantly the importance of the interactions between charged groups in the S-peptide, implying that the bulk dielectric constant is not the important factor. It is possible that the charged-group effect is one of many interactions affecting α-helix stability. At 0°C, the TFE titration appears cooperative. Since this cooperativity depends on temperature, σ and s must vary in some way with temperature in these solvents. However, we cannot say what the nature of these changes are with the present data. Finally, TFE appears to preferentially stabilize parts of the α-helix, presumably up to residue 13, thus recognizing the helix stop signal. We must be cautious with this last conclusion, however, based solely on these data.

The final question concerns the suitability of TFE as a probe for studying marginally stable structures. We can list four important criteria for a useful structure stabilizer: 1) The transition from coil to helix should be cooperative as the stabilizer concentration is increased; 2) the importance of one type of interaction, such as the effects of charged groups, should not be predominately amplified; 3) inherently more stable structures should be stabilized more than less stable structures; and 4) the stabilized structure should be the same as that formed in the absence of the stabilizer. Although the present studies do not unequivocally establish the utility of TFE as a stabilizer, the results certainly encourage us to continue with such studies.

The next step will be to obtain a more detailed description of the transitions, by using high-resolution NMR, for example, and to study other model

peptides in order to determine the generality of the TFE stabilization. Also, it would be useful to study other structure-forming solvents in order to check the consistency of the procedure. These further studies would then tell us the feasibility of determining relative stabilities of different peptides by this method.

ACKNOWLEDGMENTS

We wish to thank Drs. Robert L. Baldwin and Peter S. Kim for providing the S-peptide samples and for many valuable discussions. This work was supported by National Institutes of Health grants GM 10840 and CA 24101 and by the U.S. Department of Energy, Office of Energy Research, under contract 82 ER 60090. J.W.N. was supported by NIH training grant CA 09485.

REFERENCES

1. Kim, P.S., Baldwin, R.L. A helix stop signal in the isolated S-peptide of ribonuclease A. Nature 307: 329–334, 1984.
2. Bierzynski, A., Kim, P.S., Baldwin, R.L. A salt bridge stabilizes the helix formed by isolated C-peptide of RNase A. Proc. Natl. Acad. Sci. USA 79: 2470–2474, 1982.
3. Shoemaker, K.R., Kim, P.S. Brems, D.N., Marqusee, S., York, E.J., Chaiken, I.M., Stewart, J.M., Baldwin, R.L. Nature of the charged-group effect on the stability of the C-peptide helix. Proc. Natl. Acad. Sci. USA 82:2349–2353, 1985.
4. Kim, P.S., Bierzynski, A., Baldwin, R.L. A competing salt-bridge suppresses helix formation by the isolated C-peptide carboxylate of ribonuclease A. J. Mol. Biol. 162:187–199, 1982.
5. Rosen, H. A modified ninhydrin colorimetric analysis for amino acids. Arch. Biochem. Biophys. 67:10–15, 1957.
6. Bates, R.G., "Determination of pH: Theory and Practice." John Wiley, chapters 7, 8, 1973.
7. Johnson, Jr., W.C. Circular dichroism and its empirical application to biopolymers. Methods Biochem. Anal. 31:61–163, 1985.
8. Brown, J.E., Klee, W.A. Helix-coil transition of the isolated amino terminus of ribonuclease. Biochemistry 10:470–476, 1971.
9. Zimm, B.H., Bragg, J.K. Theory of the phase transition between helix and random coil in polypeptide chains. J. Chem. Phys. 31:526–535, 1959.
10. Sueki, M., Lee, S., Powers, S.P., Denton, J.B., Konishi, Y., Scheraga, H.A. Helix-coil stability constants for the naturally occurring amino acids in water. 22. Histidine parameters from random poly[(hydroxybutyl)glutamine-*co*-L-histidine]. Macromolecules 17:148–155, 1984.
11. Shoemaker, K.R., Kim, P.S., York, E.J., Stewart, J.M., Baldwin, R.L. Effects of N-terminal charge on helix stability in C-peptide Analogs. Unpublished manuscript.
12. Rico, M., Gallego, E., Santoro, J., Bermejo, F.J., Nieto, J.L., Herranz, J. On the fundamental role of the Glu 2−. . . Arg 10^+ salt bridge in the folding of isolated ribonuclease A S-peptide. Biochem. Biophys. Res. Commun. 123:757–763, 1984.
13. Filippi, B., Borin, G., Marchiori, F. The influence of amino acid side-chains on α-helix stability: S-peptide analogues and related ribonucleases S′. J. Mol. Biol. 106:315–324, 1976.
14. Filipi, B., Borin, G., Moretto, V., Marchiori, F. Conformational properties of the N-terminal residues of S-peptide. II. The guanidine hydrochloride-water-trifluoroethanol system. Biopolymers 17:2545–2559, 1978.
15. Rico, M., Santoro, J., Bermejo, F.J., Herranz, J., Nieto, J.L., Gallego, E., Jiménez, M.A. Thermodynamic parameters of the helix-coil thermal transition of ribonuclease-S-peptide and derivitives from ^{1}H-NMR data. Biopolymers 25:1031–1053, 1986.
16. Llinás, M., Klein, M.P. Charge relay at the peptide bond. A proton magnetic resonance study of solvation effects on the amide electron density distribution. J. Am. Chem. Soc. 97:4731–4737, 1975.
17. Toniolo, C., Bonora, G.M., Salardi, S., Mutter, M. Linear oligopeptides. 57. A circular dichroism study of α-helix and β-structure formation in solution by homooligo-L-methionines. Macromolecules 12:620–625, 1979.
18. Greenfield, N., Fasman, G.D. Computed circular dichroism spectra for the evaluation of protein conformation. Biochemistry 8:4108–4116, 1969.