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Trifluoroethanol effects on helix propensity and electrostatic interactions in the helical peptide from ribonuclease T_1

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Abstract

Trifluoroethanol (TFE) is often used to increase the helicity of peptides to make them usable as models of helices in proteins. We have measured helix propensities for all 20 amino acids in water and two concentrations of trifluoroethanol, 15 and 40% (v/v) using, as a model system, a peptide derived from the sequence of the α-helix of ribonuclease T_1. There are three main conclusions from our studies. (1) TFE alters electrostatic interactions in the ribonuclease T_1 helical peptide such that the dependence of the helical content on pH is lost in 40% TFE. (2) Helix propensities measured in 15% TFE correlate well with propensities measured in water, however, the correlation with propensities measured in 40% TFE is significantly worse. (3) Propensities measured in alanine-based peptides and the ribonuclease T_1 peptide in TFE show very poor agreement, revealing that TFE greatly increases the effect of sequence context.

Keywords: α-helix; helical propensity; peptide; ribonuclease T_1; TFE; trifluoroethanol

The tendency of some short peptides to form helical structure in solution has led to their use as models for protein folding and stability. These include synthetic peptides of de novo design (some recent reviews: Chakrabartty & Baldwin, 1995; Scholtz & Baldwin, 1995; Kallenbach et al., 1996), and protein fragments (e.g., Goodman & Kim, 1989; Waltho et al., 1993; Myers et al., 1996). Structured peptides can serve as models for investigating the contribution of local interactions to protein folding and stability (Muñoz & Serrano, 1996; Myers et al., 1996).

2,2,2-Trifluoroethanol (TFE) is a co-solvent that has been shown to stabilize helical structure in peptides (Goodman et al., 1963; Nelson & Kallenbach, 1986; Jasanoff & Fersht, 1994; Cammers-Goodwin et al., 1996; Luo & Baldwin, 1997 and references therein). Peptide fragments of protein helices often show little helix formation in water, and TFE is frequently used to induce helix formation (Sonnischen et al., 1992; Hamada et al., 1995; Kemmink & Creighton, 1995; Yang et al., 1995; Bolin et al., 1996). Although its use is widespread, the mechanism of TFE stabilization of secondary structure is not clear. One important question is whether helix propensities of the amino acids measured in water still apply in aqueous TFE solutions. This question has been addressed recently in alanine-based peptides by Baldwin and co-workers (Rohl et al., 1996; Luo & Baldwin, 1997). For reasons that are not completely clear, in water the helix propensities are twice as large in alanine-based peptides than in peptides based on natural protein sequences. Because TFE is used so widely, it is important to compare its effect on helix formation in natural peptide sequences.

Recently, we have developed a variant of the small protein ribonuclease T_1 (RNase T_1) and a peptide corresponding to the single α-helix of RNase T_1 as a model system. We have used the T_1 peptide/protein system previously to compare directly helix propensities in peptides and proteins (Myers et al., 1997a, 1997b), and to investigate the effects of other types of mutations on helix stability in the peptide and protein (Myers et al., 1996). Here, we report measurements of helix propensity with the T_1 peptide in two concentrations of trifluoroethanol, and compare the results with those found in water. We also compare our results with those found in alanine-based peptides at the same concentration of TFE.

Results

The sequence of the peptide used in this study is

S S D V S T A Q **A A** A Y K L H E D

13 21 29

which corresponds to the α-helix of ribonuclease T_1 (residues 13–29), except that the underlined alanine has been changed from a

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glycine in order to increase the helicity of this peptide (Myers et al., 1996, 1997a) and the N and C termini are blocked with acetyl and carboxamide groups, respectively. The far-UV CD spectrum of this Gly 23 to Ala peptide is characteristic of a mixture of α-helical and random coil structures (not shown). It shows a fractional helicity of 15% at pH 7.0 and 30% at pH 2.5; the helix content is independent of peptide concentration. We refer to this host peptide as wt*.

Twenty peptides with all of the amino acids at position 21 (labeled in boldface in the sequence above) were synthesized. The helical content of these peptides was measured using CD spectroscopy to determine the mean residue ellipticity at 222 nm. Fraction helix values for the peptides in water at pH 7.0 and 2.5 are shown in Figure 1. The mean residue ellipticity of the 20 peptides was also measured in two concentrations of TFE, 15% (v/v) and 40% (v/v), at both pH 7.0 and 2.5 (Fig. 1). TFE substantially increased the fractional helicity of all the peptides. In 15% TFE, the helicity ranges from 18 to 46% (average = 32%) at pH 2.5, and from 9 to 29% (average = 20%) at pH 7.0. In 40% TFE, the helicity ranges from 42 to 64% (average = 53%) at pH 2.5, and from 34 to 64% (average = 50%) at pH 7.0. When this is compared to the helicity in water, 9 to 30% (average = 18%) at pH 2.5 and 5 to 15% (average = 10%) at pH 7.0, one can see the typical stabilization of helical structure by TFE.

The other principal observation from the data in Figure 1 is the difference in helicity of the peptides at neutral and acidic pH. Although the peptides were almost twice as helical at pH 2.5 than at pH 7.0 in water, this pH dependence is reduced in 15% TFE and almost eliminated in 40% TFE. This is evident from the slopes given in Figure 1, which were determined using only the residues that do not change ionization state between pH 7.0 and 2.5 (the solid symbols). Because there are large differences in helix contents for nearly all the peptides, the pH effects must be on the stability of the host peptide. We have previously demonstrated that the ionization of Glu 28 and Asp 29 in the RNase T_1 peptide are almost completely responsible for the pH-dependence of helicity in the host peptide (Myers et al., 1996). The charged forms of the two acidic residues at the C-terminus make unfavorable interactions with the helix macrodipole; this interaction is also observed in the protein (Walter et al., 1995; Myers et al., 1996). Curiously, the addition of TFE diminishes these unfavorable interactions and the pH dependence of helicity is lost in 40% TFE (Fig. 1C).

Relative free energy changes, $\Delta(\Delta G)$, were determined for the peptides from the fractional helicity values using the Lifson–Roig model for the helix to random coil transition. These $\Delta(\Delta G)$ values represent helix propensity relative to alanine at residue 21 in the wt* peptide (Table 1). In Figure 2, we have averaged the $\Delta(\Delta G)$ values for the pH 2.5 and pH 7.0 data for all the residues that do not change ionization state over this pH range. Figure 2A shows a comparison between the water and 15% TFE data sets. The two sets are highly correlated (R = 0.92), and the slope of the best-fit line is 0.92, indicating the overall propensity differences are slightly smaller in TFE. A comparison of $\Delta(\Delta G)$ measured in water and in 40% TFE is shown in Figure 2B. The two data sets are poorly correlated (R = 0.54) and the slope of the best-fit line is only 0.44, indicating that the range of propensities is smaller, continuing the trend seen in 15% TFE. Together, the data shown in Figure 2 clearly demonstrate that the helix propensities of the amino acids do not increase uniformly in TFE and that helix propensity in 40% TFE solution bears little resemblance to helix propensity in water.

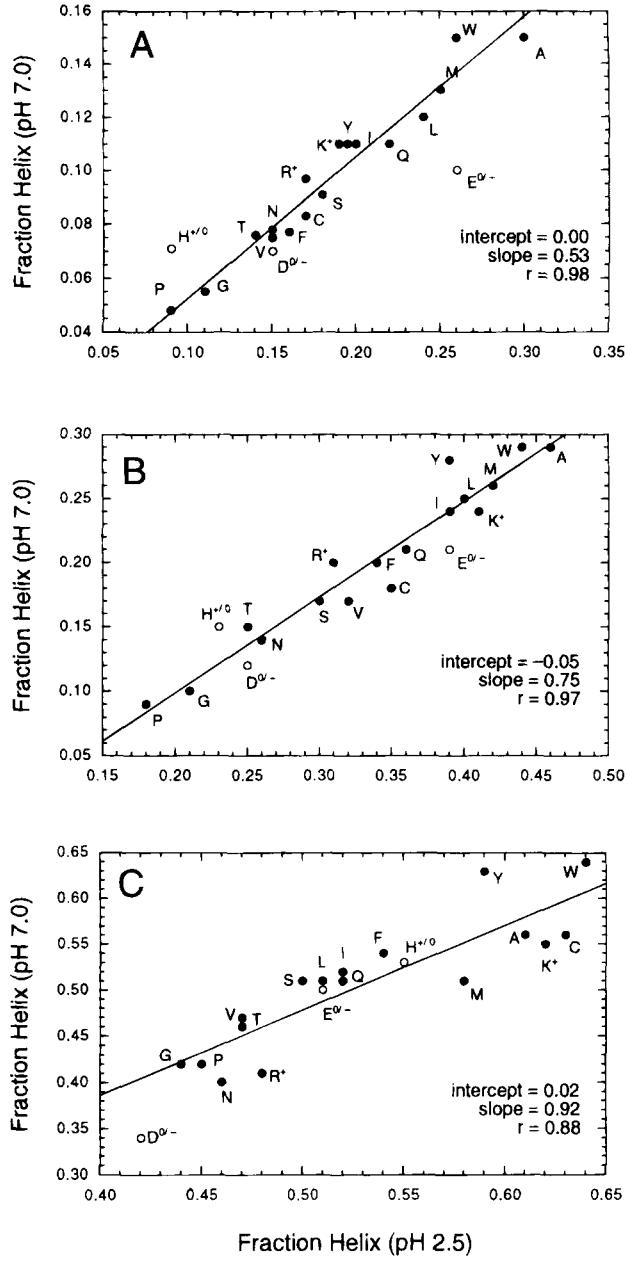

Fig. 1. Comparison of the fractional helicity of the peptides with the indicated residue in (**A**) 0% (v/v) TFE, (**B**) 15% (v/v) TFE, and (**C**) 40% (v/v) TFE. Residues that change ionization state between pH 2.5 and 7.0 are shown as open symbols and are excluded from the correlation line. The CD data and the measured fractional helicity data used to construct these plots can be found in the supplementary material that accompanies this paper.

Discussion

There are many factors that contribute to helix formation, including the intrinsic propensity of the individual residues to adopt helical structure, specific interactions between side chains (both polar and nonpolar interactions), and nonspecific interactions between charged side chains and the helix macrodipole (reviewed in Scholtz & Baldwin, 1992, 1995; Chakrabartty & Baldwin, 1995; Kallenbach et al., 1996). Many of these properties and factors are

Table 1. *Relative helix propensities, Δ(ΔG) in kcal mol^{-1}, for amino acids in 0, 15, and 40% TFE at acidic and neutral pH*[a]

	pH 2.5			pH 7.0		
Residue	0% TFE	15% TFE	40% TFE	0% TFE	15% TFE	40% TFE
A (wt*)	0	0	0	0	0	0
C	0.53	0.37	−0.11	0.51	0.43	0.00
D	0.66	0.71	0.68	0.68	0.82	0.72
E	0.17	0.24	0.39	0.31	0.32	0.20
F	0.61	0.38	0.30	0.59	0.34	0.07
G	0.98	0.91	0.64	0.95	0.98	0.46
H	1.20	0.84	0.27	0.67	0.64	0.09
I	0.38	0.23	0.35	0.29	0.16	0.14
K	0.45	0.18	−0.06	0.30	0.17	0.00
L	0.25	0.21	0.38	0.19	0.12	0.17
M	0.18	0.13	0.12	0.12	0.09	0.17
N	0.66	0.69	0.56	0.58	0.66	0.52
P	1.10	1.10	0.58	1.10	1.20	0.45
Q	0.31	0.32	0.37	0.29	0.30	0.17
R	0.56	0.51	0.50	0.38	0.38	0.49
S	0.51	0.56	0.42	0.42	0.50	0.16
T	0.71	0.72	0.52	0.59	0.66	0.32
V	0.66	0.46	0.51	0.61	0.48	0.28
W	0.14	0.06	−0.13	0.02	−0.02	−0.43
Y	0.45	0.22	0.07	0.31	0.01	−0.33

[a]Change in free energy of helix formation, determined using the Lifson–Roig helix to random coil model as described in the Materials and methods from the measured helicity of the peptides. For each solution condition (pH or %TFE), the free energies are expressed relative to Ala.

expected to change as the nature of the solution changes, as it does upon the addition of TFE. Here we are concerned principally with comparing changes in helical propensity in water and aqueous TFE, but our results also shed some light on changes in the other helix-stabilizing interactions in aqueous alcohol solutions.

TFE is a highly polar molecule, with enhanced hydrogen bond accepting capability and decreased hydrogen bond donating capability relative to water (Llinas & Klein, 1975). The dielectric constant of TFE is much lower than water, 27 and 79, respectively (Nelson & Kallenbach, 1986), therefore, TFE solutions will have a lower dielectric constant than pure water and electrostatic effects should be enhanced. However, earlier studies on the role of electrostatic interactions in the stability of the S-peptide from RNase A have shown that magnitude of the charged group effects is unaltered in the presence of TFE (Nelson & Kallenbach, 1986, 1989). A general mechanism by which TFE and other aliphatic alcohols could stabilize helices has been suggested (Conio et al., 1970; Storrs et al., 1992; Cammers-Goodwin et al., 1996). These authors suggest that TFE raises the free energy of the random coil state, because TFE–water mixtures are less able to solvate the amide group of the peptide backbone. Therefore, TFE favors states where the backbone amide groups form intramolecular hydrogen bonds, such as the α-helix.

We find that TFE does indeed increase the amount of helical structure in all of our peptides (Fig. 1 and supplementary material in Electronic Appendix), however, the pH dependence of the amount of helical structure in the RNase T_1 peptides changes with TFE concentration. In water, all our peptides are roughly twice as helical at pH 2.5 as at pH 7.0 (Fig. 1A). This has been shown to be due to the interaction between the side chains of Glu 28 and Asp 29

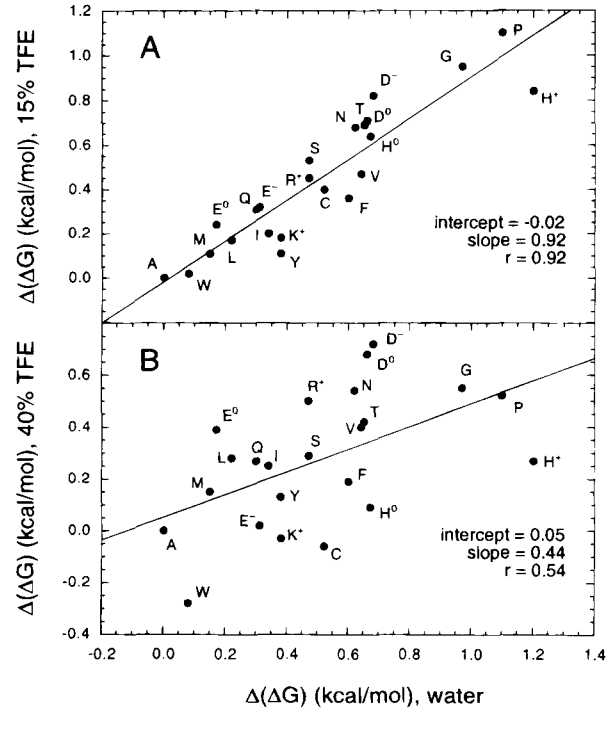

Fig. 2. Comparison of helix propensity, $\Delta(\Delta G)$ relative to alanine, measured in the RNase T_1 peptide in water and (**A**) 15% (v/v) TFE, (**B**) 40% (v/v) TFE. For the residues that do not change ionization state between pH 2.5 and 7.0, the $\Delta(\Delta G)$ value represents the average of the two values at acidic and neutral pH shown in Table 1. Solid lines are best-fit linear regressions and the intercepts, slopes, and correlation coefficients are shown.

and the helix macrodipole (Myers et al., 1996). At neutral pH, the charged side chains interact unfavorably with the macrodipole of the helix, resulting in lower helicity for the peptide. By 40% TFE, the peptides are just as helical at pH 7.0 as they are at pH 2.5 (Fig. 1C). This is not due to the fact that we have reached 100% helix in the peptides, because we still find a substantial difference between the helix contents in our peptides at 40% TFE. (The range of helix contents found is 34–64%, indicating that we have not reached maximum helix formation in any of the peptides.) The logical conclusion is that we have either diminished the interaction between the helix macrodipole and the charged forms of Glu 28 and Asp 29, or that we have compensated for the unfavorable charge–macrodipole interactions by strengthening other interactions in the host peptide. It is known that hydrophobic interactions are magnified in TFE and that these can contribute to helix stabilization (Albert & Hamilton, 1995); however, we would need to invoke a pH-dependent change in the strength of the hydrophobic interactions in order to reconcile our results. Another alternative is that the pK_a values for Glu and Asp are highly elevated in 40% TFE, above pH 7. We are currently testing some of these possibilities with other variants of our RNase T_1 peptide.

The relative helix-forming tendency of all the amino acids does increase in TFE, although not uniformly. This is easily observed in Figure 3. Here we have set the ΔG for helix formation for Gly in water to zero and calculated $\Delta(\Delta G)$ for all other residues in water and in the two TFE solutions relative to the value for glycine in water. If TFE altered helix propensity uniformly, we would expect the 15% and 40% TFE values to be offset by a constant amount for all residues and for the rank order of propensities to remain unchanged. In 15% TFE, this appears to be roughly what is observed: the peptides are about twice as helical as they are in water (Fig. 1) and the rank order of propensities remains the same as that found in water. However, in 40% TFE, the peptides are roughly five times as helical as they are in water (Fig. 1), but the rank order of helix formers and the relative differences between the residues is clearly different. Thus, we find a reasonable correlation between helix propensity in 15% TFE and water and this correlation is clearly lost by 40% TFE (Fig. 2). Consequently, our results suggest that solutions containing lower concentrations of TFE (15% TFE) may mimic helix formation in water, but it is questionable to draw deductions about helix formation in water based on data obtained in higher concentrations of TFE (like 40% TFE).

A comparison of the propensities measured with the RNase T_1 peptide in 40% TFE and those measured with alanine-based peptides under identical conditions (Rohl et al., 1996) is shown in Figure 4. There is very poor correlation between the two data sets in contrast with the comparison of the two systems in water, where reasonable qualitative agreement was found (Myers et al., 1997b). In the alanine-based peptides (Rohl et al., 1996), the helix propensities for the nonpolar residues in 40% TFE correlate well with the differences in side-chain conformational entropy (Blaber et al., 1994; Creamer & Rose, 1994; Wang & Purisma, 1996). If, however, only the nonpolar residues with side chains (Ala, Ile, Leu, Met, Phe, and Val) are considered, the correlation between the alanine-based peptides and the T_1 peptides is not improved. Thus, even for the simplest residues, the nonpolar, non-glycine residues, the alanine-based peptides and the T_1 peptides are not in good agreement. This suggests that TFE has differential effects on the "host" sequence and that the effect of TFE on helix formation is context dependent. Therefore, it seems likely that solvation differences play an important role in determining both the rank order and magnitude of helix propensities in 40% TFE. A similar conclusion was reached in comparing the two peptide systems in water (Myers et al., 1997a, 1997b).

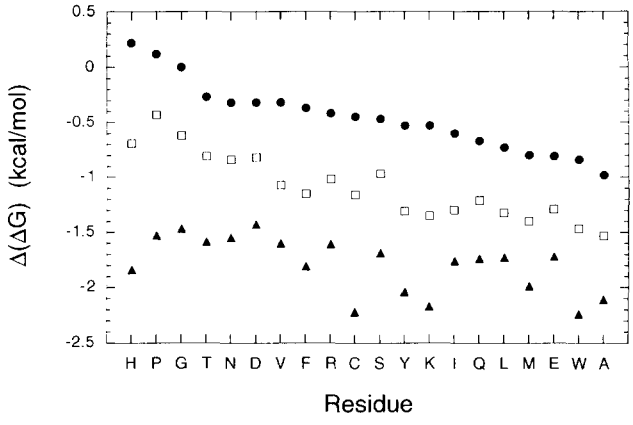

Fig. 3. Differences in helix propensity are shown for each residue in water (●), 15% TFE (□), and 40% TFE (▲). For this plot, the scale is set relative to the value for glycine in water and the residues are ordered relative to glycine in water. A value of $\Delta(\Delta G)$ less than zero is helix stabilizing. TFE increases helix propensity, but not in a uniform manner (see text).

Our comparison of propensity scales in water revealed that reasonable qualitative agreement exists between propensities measured in various systems (Myers et al., 1997a, 1997b). This allowed an experimentally based consensus scale of helix propensities to be derived (Pace & Scholtz, 1997). The poor correlation for the polar residues between alanine-based peptides and the RNase T_1 peptides in 40% TFE might indicate that it will be difficult to find a consensus propensity scale in TFE solutions. We have argued that backbone solvation is a key factor in propensity differences in water and that different host systems will necessarily differ in solvation properties. Unexpectedly, the solvation differences appear to be exaggerated in 40% TFE solutions. Our finding that electrostatic interactions are significantly reduced in 40% TFE solutions is consistent with this interpretation. Therefore, helical propensities, polar, and electrostatic interactions depend on both

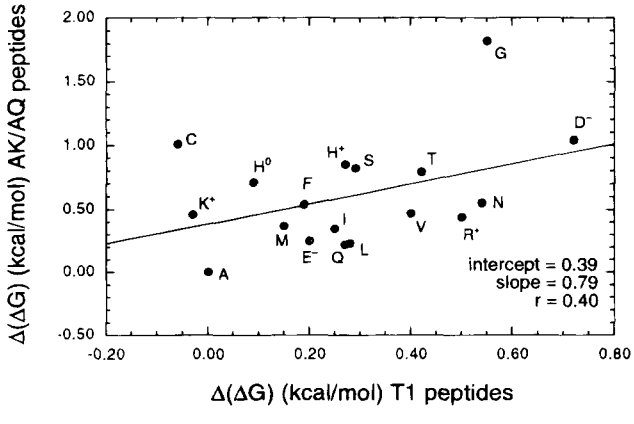

Fig. 4. Comparison of helix propensity $\Delta(\Delta G)$ relative to alanine measured with the RNase T_1 peptide to the results from the alanine-based peptides (Rohl et al., 1996) under identical conditions of 40% (v/v) TFE. The solid line is a best-fit linear regression for the data.

TFE concentration and, more importantly, on the structure of the individual peptide.

Conclusions

In 15% TFE, we find reasonable agreement with helix propensities in water and thus 15% TFE solutions may be useful for determining conformational preferences for peptides. However, TFE does not increase helical propensity for all amino acids uniformly, and propensity scales measured in water are not applicable to 40% TFE solutions. Additionally, 40% TFE dramatically alters electrostatic (and polar) interactions in the RNase T_1 peptides and increases the dependence of helix propensities on the sequence of the host peptide. Thus, caution is needed in drawing conclusions about the energetics of helix formation in water from studies in 40% TFE solutions.

Materials and methods

Peptides were synthesized, purified, and characterized as described previously (Myers et al., 1997a, 1997b). The helix content of the peptides was measured using an Aviv model 62DS CD spectropolarimeter. Each 1-mL sample contained 30 μM peptide in CD buffer (1 mM each of potassium phosphate, borate, and citrate) in a 0.5-cm pathlength cuvette, maintained at 0 °C by a built-in temperature controlling unit. TFE was from Sigma or Aldrich and was used without further purification. All TFE concentrations are reported as volume percent (% v/v). For samples containing TFE, the pH was adjusted according to the procedure described previously (Nelson & Kallenbach, 1986). The cysteine-containing peptide was measured in CD buffer containing 10 mM DTT to inhibit intermolecular disulfide formation. Peptide stock solutions were made in water and the peptide concentration was determined by UV absorbance, using extinction coefficients at 276 nm of 1,390 M^{-1} cm^{-1} for tyrosine, and 5,455 M^{-1} cm^{-1} for tryptophan (Pace et al., 1995). The CD signal at 222 nm (in millidegrees), after subtracting the blank signal of cuvette and buffer (Δm°), was converted to mean residue ellipticity (in deg cm^2 $dmol^{-1}$) using the equation:

$$[\theta]_{obd} = \frac{100 \cdot \Delta m^\circ}{C \cdot n \cdot l}, \quad (1)$$

where C is the peptide concentration in mM, $n = 17$ (the number of residues in the peptide), and l is the pathlength in cm. This ellipticity ($[\theta]_{obs}$) was converted to fraction helix values using

$$f_H = \frac{[\theta]_{coil} - [\theta]_{coil}}{[\theta]_{helix} - [\theta]_{coil}}, \quad (2)$$

where $[\theta]_{helix}$ and $[\theta]_{coil}$ represent the mean residue ellipticity of a complete helix $[-42{,}500 \cdot (1 - (3/n)]$, where n = number of residues in the peptide) and complete random coil (+640), respectively (Rohl et al., 1996).

To convert the measured helicities into a free energy scale, we used the homopolymer version of the standard Lifson–Roig helix to random coil model (Lifson & Roig, 1961) as described previously (Qian, 1993). The wt* peptide is defined as the host peptide and all the other peptides contained guest residues at position 21. The nucleation constant, v^2, was taken to be 0.0023 (Rohl et al., 1992). By treating the host peptide as a homopolymer, we can assign one w value to the whole peptide based on the measured helicity. The w value is the helix propagation parameter, and represents the equilibrium of a residue between a random coil and a helical conformation. The w values of the guest residues were determined from the fractional helicity of the respective peptides. Free energy changes can be calculated using $\Delta G = -RT \ln[w/(1 + v)]$. Relative changes in ΔG were calculated using $\Delta(\Delta G) = -RT \ln(w_{guest}/w_{host})$, so that a positive $\Delta(\Delta G)$ indicates destabilization of the helix.

Supplementary material in electronic appendix

One table showing the helical contents of all peptides used in this study can be found in the Electronic Appendix.

Acknowledgments

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