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Lifson–Roig nucleation for α-helices in trifluoroethanol: context has a strong effect on the helical propensity of amino acids

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Abstract

We have investigated the effect of substituting each of 19 common amino acids (excluding P) at the X position in the peptide acetyl-Y-VAEAK-TSXSR-VAEAK-NH_2. This very different peptide is of interest because, in previous work, we showed that nucleation in the strong α-helix-forming pentamers VAEAK was unable to propagate the helix structure through the sequence TSDSR, which is neither a helix-forming sequence nor a breaker, but is indifferent to helix formation. Substitution in the center of the indifferent sequence reveals an interesting measure of the helical propensity for the 19 amino acids. CD spectra were measured in various mixtures of buffer and 2,2,2-trifluoroethanol (TFE), and then analyzed for helix propensity of the amino acids using the Lifson–Roig model. However, the nucleation parameter in the Lifson–Roig model has never been measured for TFE. We have empirically found that the nucleation parameter for a solvent can be determined from the data normally used to determine only the propagation parameters. The results of the analysis of the CD show that most amino acids are excellent or good helix formers in 90% TFE, while amino acids D, W, F and G are poor helix formers for the indifferent pentamer sequence. The helix propensity of the 19 amino acids is quite different from the helix propensity measured in other peptide sequences, demonstrating the context dependence of this property. The results as a function of alcohol concentration confirm that the relative order of helical propensity of amino acids changes with solvent environment. Clearly, the prediction of α-helical secondary structure from protein sequence requires more than a single helical propensity for each amino acid.

Keywords: α-Helix; Propensity; Context; Lifson–Roig

1. Introduction

The ability to synthesize amino acid sequences has prompted a number of laboratories to experimentally determine the α-helical propensity of the common amino acids [1–20]. Initial guest–host studies gave similar propensities for the various amino acids, but more recent studies show that the propensity of an amino acid to form an α-helix depends on the identity of its amino acid neighbors. This context dependence for α-helical propensity has been discussed by Kallenbach and co-workers [18], and illustrated by a comparison

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of previous guest–host work from many laboratories in an earlier publication [20]. This may well be due to side chain interactions, charges, or other factors not well understood.

Most studies of α-helical propensity have been carried out in aqueous solution, [1–8,11–13,15–18], but some have been carried out in mixed solvents [14,19,20]. The studies in mixed solvents show that, in addition to context dependence, the relative order of helical propensity of amino acids changes with solvent environment.

In earlier work [21], our laboratory compared the sequence VAEAK, with a high helical propensity, to the sequence TSDSR. The sequence TSDSR represents a conservative substitution of the amino acids in VAEAK, and each of these amino acids is indifferent to helical formation by the Chou–Fasman criteria, but are not helix breakers. The 15-mer peptide formed by taking the pentamer VAEAK three times and blocking the ends with acetyl and NH_2 groups easily forms an α-helical structure at 2,2,2-trifluoroethanol (TFE) concentrations of 40% and above. In contrast, the blocked 15-mer peptide formed by taking the pentamer TSDSR three times remains a random coil, even at 80% TFE. We investigated blocked 15-mer peptides that were mixtures of VAEAK (H) and TSDSR (I) in all possible permutations [21]. One model for the formation of α-helical structure postulates an initial nucleation involving a few neighboring residues with a strong propensity for the secondary structure, and then propagation of the structure, even through indifferent residues, until strong breakers terminate the growth on both ends. The Zimm–Bragg and Lifson–Roig models [22] make use of the nucleation–propagation idea. We expected that our nucleation sequence would propagate an α-helical structure through the indifferent sequence. However, this did not prove to be the case. The amount of α-helical structure at 80% TFE was approximately proportional to the amount of nucleation sequence, H. Even the peptide HIH, with the indifferent sequence sandwiched between two nucleation sequences, showed only two-thirds the α-helical structure of sequence HHH at 80% TFE. Nucleation did not appear to propagate α-helical structure through the indifferent sequence.

The peptide HIH offers an interesting opportunity to measure the helical propensity of the amino acids in a very different peptide. The host peptide of sequence acetyl–Y–VAEAK–TSXSR–VAEAK–NH_2, where the guest X is substituted with one of 19 amino acids (P excluded), was titrated with TFE to study the ability of each of these amino acids to cause the entire 16-mer to form an α-helix. We found that the residues D, W, F, and G are poor helix formers. S, A, C, I, M, K, V, and N are all excellent helix formers in 90% TFE, confirming that the indifferent sequence with D, W, F and G as X is the part of the peptide that is not α-helical. The order for the 19 amino acids is quite different from previous measurements using other peptides. This confirms that context has a strong effect on the helical propensity of the amino acids.

The Lifson–Roig statistical model was used to calculate the free energy for helix propagation, but the nucleation parameter for water [13] ($v=0.048$) turns out to be too large to be used with high concentrations of TFE. Empirically, we found that amino acids that are excellent helix-formers give a minimum in their helix propagation parameter (w) as a function of v at what appears to be the correct value of v.

2. Materials and methods

2.1. Peptide synthesis

The host peptides acetyl–Y–VAEAK–TSXSR–VAEAK–NH_2, where guest X is substituted with each of the 19 naturally occurring amino acids (excluding P), were synthesized by TANA Laboratories in Houston, Texas. The crude peptides were purified by reverse-phase high-performance liquid chromatography using a Vydac C-18 reverse-phase semipreparative column in a Hewlett Packard 1040 HPLC instrument. The hydrophobic gradient was 0.1% trifluoroacetic acid/water and 0.1% trifluoroacetic acid/acetonitrile at 2% min^{-1}. The fractions were collected according to the absorption of the Y tag at 280 nm and the

amide bond at 214 nm, and were then lyophilized and stored in the freezer until needed. The molecular weight for all peptides was confirmed by fast atom bombardment.

2.2. Circular dichroism measurements

Circular dichroism (CD) measurements were performed using a Jasco J-720 spectropolarimeter (Jasco Inc.). The instrument was purged with nitrogen gas at 20 l min^{-1} for 30 min before and during measurements. A two-point calibration with (+)-10-camphorsulfonic acid gave a ratio between 2.0 and 2.1 for the magnitude of the 192.5- and 290.5-nm bands [23]. Since the α-helical content of the peptides increases with lower temperature, all titrations were carried out at 2 °C, using a thermoelectric constant-temperature controller. A stock solution of peptide in 10 mM sodium phosphate buffer, pH 7, was prepared in 18-MΩ distilled water. Stock solution, 18-MΩ water, and NMR-grade trifluoroethanol (TFE) were kept on ice, along with a microcentrifuge tube that had been pierced through the top by a syringe needle. This helped to prevent the TFE from evaporating. A small volume of stock solution was added to the tube, then appropriate amounts of water and TFE were slowly introduced while vortexing to prepare the sample solution. The final buffer concentration was 1 mM. The tube was closed at all times to avoid evaporation and sealed with parafilm while waiting for 10 min at room temperature to reach equilibrium. This procedure was repeated with increasing amounts of TFE at 10% increments, resulting in TFE concentrations of 0–90%. Each sample was then transferred to a 1-mm rectangular quartz cell (Hellma Cells Inc) and a temperature probe was introduced. When the temperature reached 2 °C, the spectra were recorded from 260 to 190 nm using a 2-nm bandwidth, 20-nm min^{-1} scan speed, 2-s response time, and three accumulations. A baseline of the empty chamber was recorded between each measurement, and the last measurement was of the last solvent in the same cell. Baselines with and without the cell were identical. Sample concentrations ranged from 0.20 to 0.47 mM in amide, and the intensity of the CD spectra is expressed as $\Delta\varepsilon$ per amide bond.

2.3. Ultraviolet absorption measurements

UV absorption measurements were performed at room temperature using a Cary 15 spectrophotometer interfaced to a computer. The machine was flushed with nitrogen gas for 30 min before and during the measurement. Absorption spectra for the amides were recorded either from 350 to 180 nm in a 100-μm cylindrical cell, or from 400 to 185 nm in the same 1-mm rectangular cell used for CD. The absorption spectra for the aromatics were recorded from 400 to 240 nm using a small-volume (500 μl) 1-cm rectangular quartz cell.

2.4. Amide concentration measurements

We measured the UV absorption of random-coil peptides in the stock solution buffer at 280 nm using 1.0-cm cells. The absorption at 280 nm and the known ε value at 280 nm of the Y tag (1280 M^{-1} cm^{-1}) plus any other aromatics or cystine (5690 M^{-1} cm^{-1} for W and 120 M^{-1} cm^{-1} for C) were used to calculate the molar concentration of peptide in the stock solutions [24]. The peptide concentration was converted to the amide bond concentration by multiplying by the 17 amide bonds. The concentration in all sample solutions is then known by dilution.

2.5. Checking for aggregation

Aggregation experiments were performed with two peptides that were deemed most likely to aggregate according to previous experiments, those with F and L substituted in the X position. The CD intensity of those peptides at 222 nm over a large range of concentrations was measured in 90% TFE solution, which is the solvent in which the peptides are least soluble and thus most likely to facilitate aggregation. The peptide concentration was varied from 3 μM to 9 mM amide, and the CD intensity of the peptides was found to be independent of the concentration. Since the concentration used in our titration experiment was

approximately 0.2–0.4 mM, we are confident that no aggregation occurred in this work. We confirmed that there was no aggregation by measuring the absorbance of all peptides in 0 and 90% TFE and observing no light scattering between 400 and 300 nm.

2.6. Correction for aromatic contribution to the CD

The aromatic and sulfur-containing side chains W, Y, F, C and M contribute to the CD spectra of peptides and proteins in the amide region, and can interfere with the analysis for secondary structure. We used the method developed in our laboratory [25] to correct the CD spectra of the peptides containing these side chains. Briefly, this method uses the common basis vectors obtained from a singular-value decomposition analysis of the CD spectra of the related peptides without aromatic or sulfur-containing side chains. The common basis vectors from singular value decomposition are fitted to a portion of the CD spectrum of the peptide being corrected, in the range that is not affected by its side chain contributions. The resulting coefficients from the fitting are then used along with the common basis vectors to regenerate the entire corrected spectrum. For a complete explanation, see [25].

The chromophoric Y attached to the end of each peptide to monitor concentration can also contribute to the CD [26]. We have shown in earlier work [20] that attaching a Y to the end of the fundamental peptide in this series, $(VAEAK)_3$, without intervening Gs does not affect the CD when $\Delta\varepsilon$ is calculated on a per amide basis.

2.7. Calculation of helical propensity

We used the Lifson–Roig statistical model [27] to calculate a free energy of propagation, ΔG^o, for each amino acid in each buffer–TFE cosolvent. The free energy of propagation is a measure of the helical propensity of the amino acid under given conditions. The model considers the helix–coil transition to be two-state in residues, and utilizes a propagation parameter (w) for each residue and a nucleation parameter (v), which is normally assumed to be the same for all residues. The value of v has only been measured for water, and is 0.048 [13]. The values of v for the water–TFE cosolvents were determined as described in Section 3. In this model, the equilibrium constant for adding a residue to a helical segment is $w/(1+v)$, so the free energy for the change in stability of the helix on adding a residue is $\Delta G^o = -RT\ln[w/(1+v)]$. We used the subroutine written by Qian and Schellman [22], which calculates the fraction each residue is in an α-helix from the given parameters. This is then compared to the fraction of residues in an α-helix measured for each peptide in its mixed solvent, as estimated by the CD intensity of the 222-nm band, $\Delta\varepsilon_{(222)}$. Propagation parameters for all the residues are varied until the calculated α-helical fraction agrees with that measured for all 19 peptides measured at a given percentage of TFE. We took $\Delta\varepsilon_{(222)}$ equal to $+1.0$ for the non-helical structure. The value of $\Delta\varepsilon_{(222)}$ for 100% α-helix for this peptide of 17 amides was taken to be -12.2 [28,29].

3. Results and discussion

CD spectra were measured for all 19 peptides of the form acetyl–Y–VAEAK–TSXSR–VAEAK–NH_2 from 260 to 190 nm at 10% (v/v) intervals of added TFE between 100% 1 mM phosphate buffer and 10% buffer–90% TFE cosolvent. A typical set of spectra for guest X as K is shown in Fig. 1. It is evident that the peptide is completely non-helical at 0% and 10% TFE. It is still very non-helical at 20% TFE, but then very co-operatively becomes helical as more TFE is added. At 70, 80 and 90% TFE the peptide is extremely helical, because of the strong helix-forming power of the TFE solvent and the low temperature of 2 °C at which the measurements were made. The magnitude of $\Delta\varepsilon$ at 222 nm was converted to ΔG^o for helix propagation of each amino acid at the X position at 30–90% TFE using the Lifson–Roig statistical model [27] for helix propagation, as detailed in Section 2. CD data for 0 to 20% TFE were not converted to ΔG^o, because the results for these weak helix-forming peptides were nearly non-helical for most

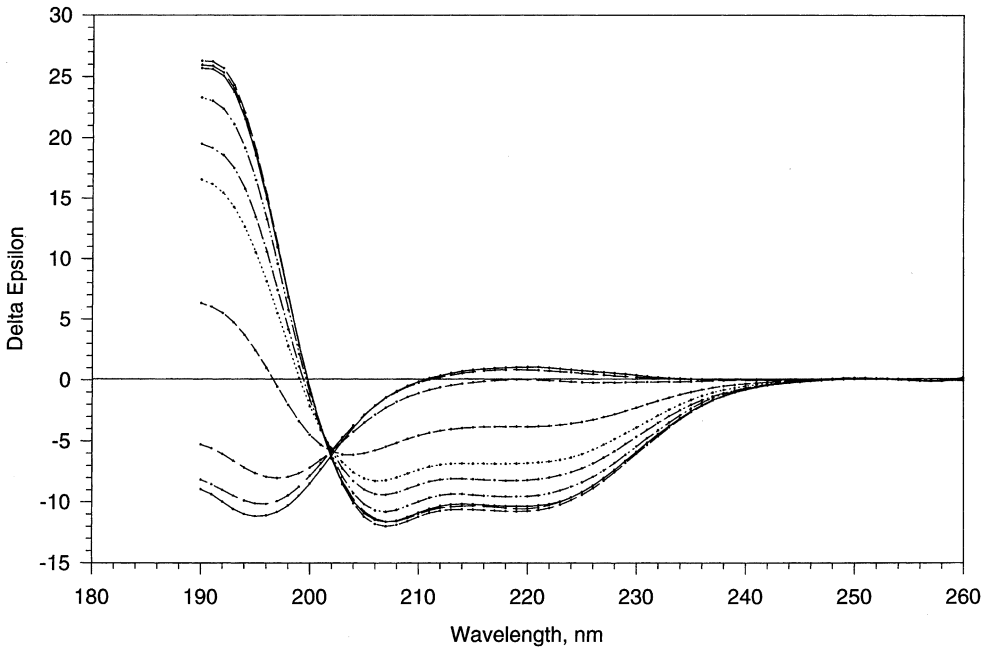

Fig. 1. The CD of acetyl–Y–VAEAK–TSKSR–VAEAK–NH_2 in various percentages of TFE. The spectrum with the most positive intensity at 222 nm corresponds to 1 mM sodium phosphate buffer, pH 7. Other spectra correspond to buffer–TFE mixtures in 10% intervals. The spectra become more negative at 222 nm as TFE is increased. The spectrum with the most negative intensity at 222 nm corresponds to 10% buffer–90% TFE.

amino acids. Plots of ΔG^o vs. 30–90% TFE for each of the 19 amino acids are given in Fig. 2.

Readers should note that we have measured the CD of the fundamental model peptide in this series, acetyl–$(VAEAK)_3$–NH_2, both with and without the N-terminal Y, in both aqueous solution and 90% TFE. The CD spectra for the two peptides are identical in shape and magnitude when $\Delta\varepsilon$ is plotted on a per amide basis. Thus, we have not added Gs between the Y and our peptides, which is sometimes necessary to avoid CD bands due to the chromophoric aromatic amino acid [26]. Furthermore, we have corrected the CD spectra of the peptides where X is an amino acid with an absorbing side chain (F, W, Y, M and C). The method for correction is presented briefly in Section 2, and in detail in a previous publication [25].

CD data measured at 0, 10 and 20% TFE is nearly completely non-helical, and thus not suitable for analysis of helical parameters. CD data at 30, 40 and 50% TFE analyze well with the Lifson–Roig model, assuming the nucleation parameter for water ($v=0.048$) applies to these cosolvents. This is in agreement with earlier work from other laboratories using 60% water–40% TFE cosolvent and the Lifson–Roig model [14,19]. However, CD data for 60, 70, 80 and 90% TFE would not analyze assuming $v=0.048$. To the best of our knowledge, no other laboratory measuring the CD of peptides in these high concentrations of TFE has used the Lifson–Roig model to analyze data. The nucleation parameter for TFE has never been measured, but on experimenting with different trial values for v, we noted that amino acids that are excellent helix formers showed a minimum in their w value as a function of v. The values of w for all amino acids at this minimum seemed most reasonable.

As an example, let us consider the cosolvent 20% buffer–80% TFE. We measured 19 CD spectra in this solvent for the 19 amino acid guests. The 19 $\Delta\varepsilon$ values we obtained at 222 nm are as

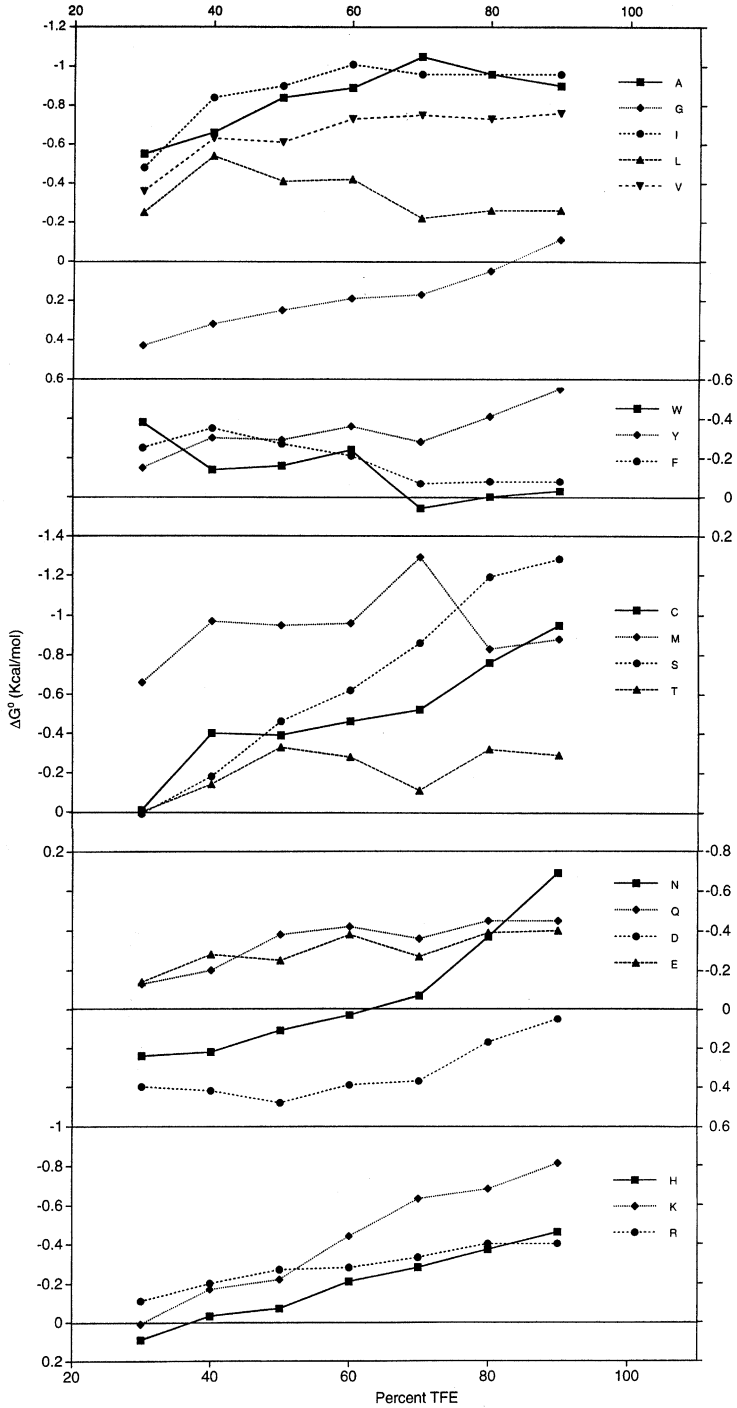

Fig. 2. The propensity of each of the 19 common amino acids (excluding proline), substituted at the X position in acetyl–Y–VAEAK–TSXSR–VAEAK–NH_2, to form an α-helix as a function of TFE. Free energy of propagation, ΔG^{o}, in the Lifson–Roig statistical model is taken as a measure of α-helical propensity. The values of v used in the data analysis are from Fig. 4, and are listed in Section 3.

follows: A, −10.80; C, −10.40; D, −7.70; E, −9.70; F, −8.80; G, −8.20; H, −9.60; I, −10.70; K, −10.30; L, −9.30; M, −10.50; N, −9.60; Q, −9.80; R, −9.70; S, −10.90; T, −9.60; V, −10.40; W, −8.40; and Y, −9.70.

We can analyze these data using a Lifson–Roig program. This takes one measurement at a time, and for a given v can calculate the w that gives the measured $\Delta\varepsilon$ at 222 nm. We did not use the homopolymer approximation, but varied the data for all 19 amino acids until they were all self-consistent. Thus, we chose a value for v, which is the same for all amino acids, and calculated 19 w values using the Lifson–Roig program.

However, if we choose $v=0.048$, the value for H_2O, the data for 20% buffer–80% TFE do not analyze. We tried various values for v, but only very small values analyzed the data. Among the values that worked were $v=0.00090$, 0.00064, 0.00045, 0.00032, 0.00023, 0.00016, 0.00008 and 0.00004. We obtained 19 values of w, one for each amino acid, for each of these eight trial values of v. For all eight sets of 19 w values, amino acid S had the largest value of w. The v,w values for S are: 0.00090, 8.39; 0.00064, 7.74; 0.00045, 7.46; 0.00032, 7.42; 0.00023, 7.45; 0.00016, 7.59; 0.00008, 7.95; and 0.00004, 8.37. Surprisingly, the value of w for S as a function of v has a minimum value at $v=0.00032$. These values are graphed in the last panel of Fig. 3.

Does this minimum have any significance? To investigate this question we used the same procedure to analyze our data published earlier [20] on the strong helix-forming peptide, acetyl–Y–$(VAXAK)_3$–NH_2, where X is any one of the 20 common amino acids except P. Using the Lifson–Roig model with various trial values of v, we found that, regardless of the concentration of methanol, the excellent helix formers gave a minimum as a function of v near the value of 0.048. The results for the strongest helix former in buffer, 45% methanol and 88% methanol are shown in Fig. 3. Furthermore, as observed in Fig. 3, 20% TFE gives a minimum near $v=0.048$ for the strongest helix former. We believe that these results are strong experimental evidence that the minimum corresponds to the true v for the solvent investigated.

Fig. 4 graphs the value of v at the w minimum for the strongest helix formers at each concentration of TFE. There is no value for 60% TFE because the strongest helix former gave no clear minimum. It is evident that high concentrations of TFE give small values of v, meaning a more cooperative transition, as expected. The graph extrapolates to approximately 0.048 for v at 0% TFE, also as expected. These are the values of the nucleation parameter that we used to analyze the CD data from our titrations: 90%, 0.00027; 80%, 0.00032; 70%, 0.00064; 60%, 0.0013; 50%, 0.0030; 40%, 0.0084; 30%, 0.028; and 20%, 0.033.

The free energy of propagation values given in Fig. 2 are a measure of the helix propensity of each amino acid as a function of the percentage of TFE. It is evident that most amino acids increase their helical propensity as the percentage of TFE is increased. Amino acids G, C, S, D, N and K show a particularly strong increase in helical propensity with increasing concentration of TFE. In contrast, the four amino acids L, W, F and M tend to decrease their helical propensity with increased percentage of TFE. Almost all amino acids have a negative ΔG^o at high concentrations of TFE. Most are extremely strong helix formers in 90% TFE (Table 1). Only D has a positive ΔG^o in 90% TFE. The plots of helical propensity with titration by TFE can cross for the various amino acids, and there is no correlation between the helical propensity at low concentrations of TFE and that at high concentrations. This confirms our earlier work, which indicated that the relative order of helical propensity of amino acids changes with solvent environment [20].

Table 1 compares the helical propensity for the 19 amino acids measured for different peptides in helix-forming mixed solvents. The helical propensity is presented as ΔG^o as calculated by the Lifson–Roig model [27], although the parameters used by different authors vary. Myers et al. [19] presented their data as $\Delta\Delta G^o$ relative to A, so we have converted their data to absolute ΔG^o by assuming a ΔG^o value for A of −0.52 [14]. The amino acids in the table are ordered according to their helical propensity for the peptide used in this study in 90% TFE. It is evident that S has by far the highest helical propensity in 90% TFE, but

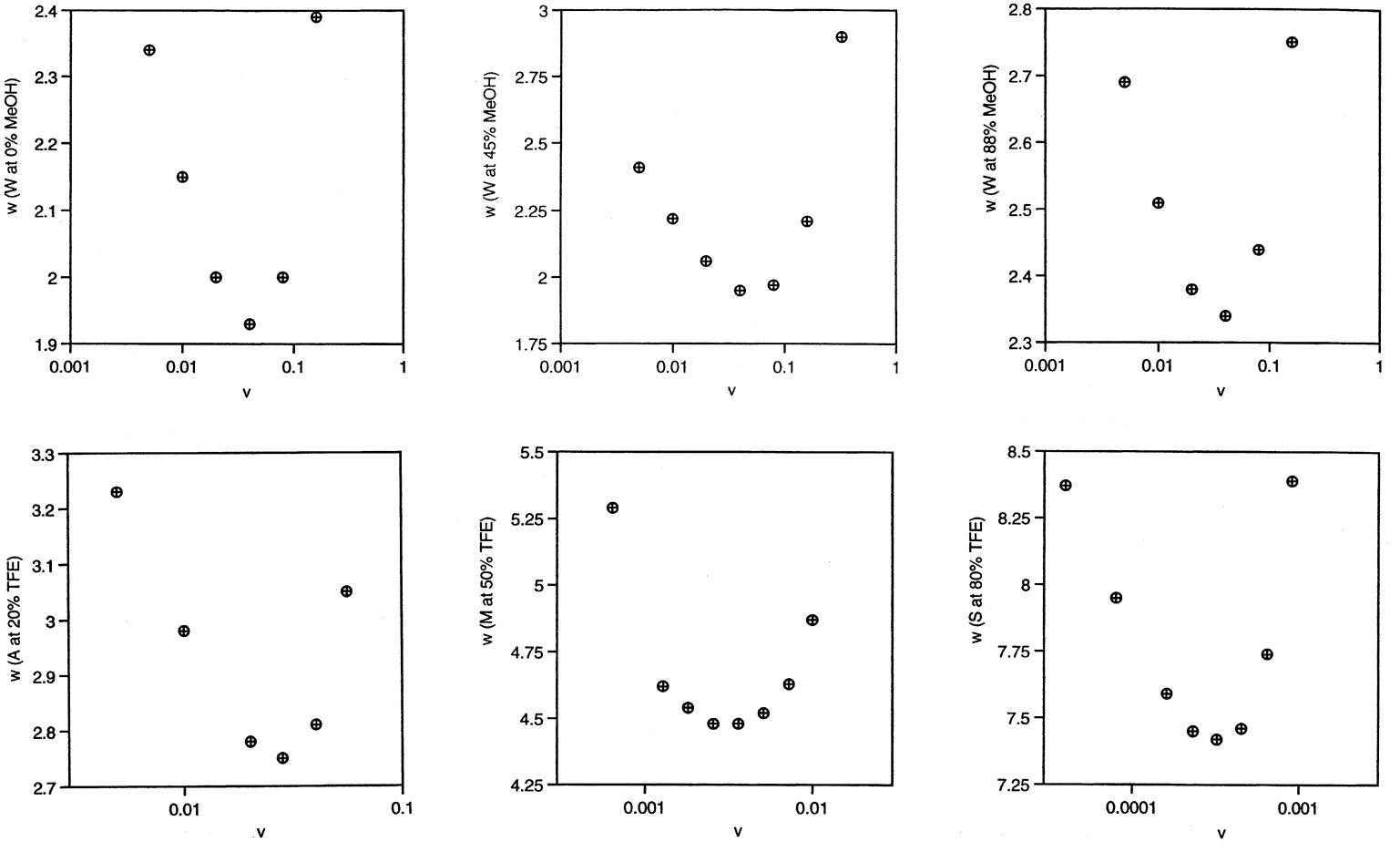

Fig. 3. Graphs of the propagation parameter (w) as a function of trial nucleation parameters (v, graphed on a log scale) as calculated by the Lifson–Roig model for the amino acid with the highest helical propensity in the solvent indicated. The host peptide for the buffer–methanol cosolvent systems was acetyl–Y(VAXAK)$_3$–NH_2 and X is W for 0, 45 and 88% methanol. The host peptide for the buffer–TFE cosolvent systems was acetyl–Y–VAEAK–TSXSR–VAEAK–NH_2 and X is A for 20%, M for 50%, and S for 80% TFE.

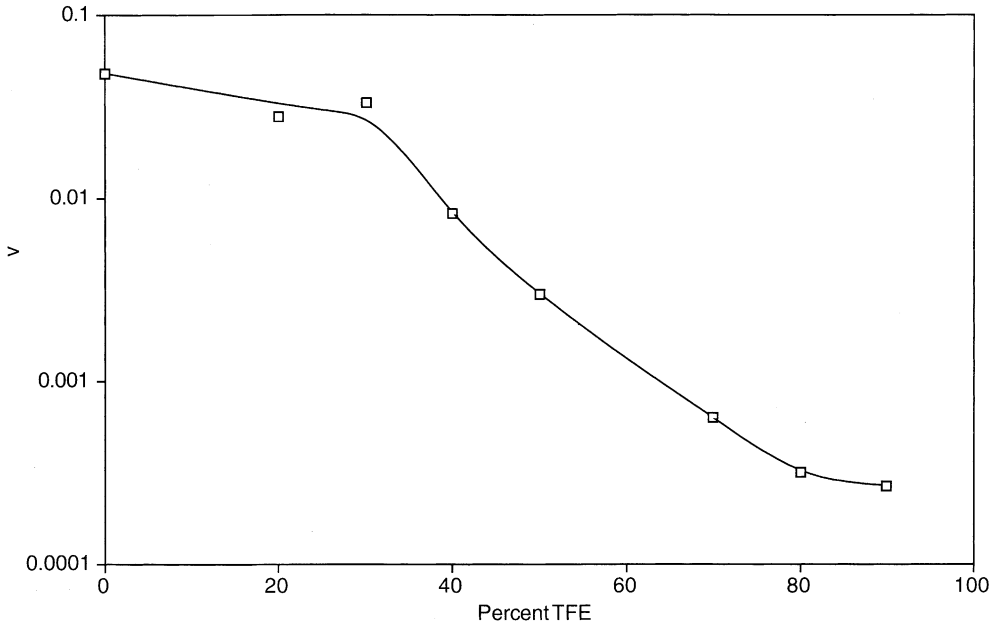

Fig. 4. Graph of the Lifson–Roig nucleation parameter (v, graphed on a log scale) as a function of TFE for buffer–TFE cosolvent systems. The values of v were determined from minima in graphs of v vs. w as calculated by the Lifson–Roig model for the amino acid with the highest helical propensity. There is no value for 60% TFE, because no clear minimum was found for the amino acid with the highest helical propensity. The value plotted at 0% TFE is the accepted value for water, $v=0.048$.

that its helical propensity markedly varies with solvent and peptide. Amino acids A, C, I, M, K, V and N also show substantial helical propensity in 90% TFE. Of these amino acids, A, I and M all show fair helical propensity in all the studies, while C, K, V and N show quite variable helical propensity in the various studies. Amino acids Y, H, Q, E, R, T and L all have a high helical propensity in 90% TFE, and their helical propensity markedly varies in the various studies shown in Table 1. Finally, G, F, W and D are all very poor helix formers in 90% TFE. G and D are poor helix formers in every study presented in the table, but F and W vary widely in their helical propensity in the various studies. Amino acid W was the best helix former for the host measured in 88% methanol, and F was the second best. All in all, Table 1 shows no correlation for the various amino acids in the five studies, indicating the strong effect of context on the helical propensity of amino acids. This may well be due to side chain interactions, charges, or other factors not well understood.

In earlier studies, workers looked for correlations between the helical propensity measured and properties, such as sidechain bulkiness, the effect of charges on the helix dipole, conformational entropy, hydrophobicity, and steric and electrostatic interactions. However, if there is a strong context dependence of helical propensity, correlations with any property of single amino acids will have little meaning, because interactions are important.

We have investigated the helical propensity for 19 amino acids in a very different peptide, in which the central five amino acids are normally indifferent to adopting a helical secondary structure, but are not helix breakers. By substituting the 19 amino acids in the center of this indifferent pentamer sequence, we find helical propensities that do not correlate well with those previously measured. Thus, this work, as well as comparisons of previous work [1–20], indicates a strong context dependence of helical propensity. This work also confirms that helical propensity depends on solvent environment. Clearly, the prediction of α-helical

Table 1
Comparison of α-helical propensities as the Lifson–Roig propagation free energy as measured by various laboratories in helix-forming mixed solvents

Residue	90% TFE[a]	40% TFE[a]	88% MeOH[b]	40% TFE[c]	40% TFE[d]
S	−1.28	−0.18	−0.19	0.30	−0.36
I	−0.96	−0.84	−0.47	−0.17	−0.46
C	−0.95	−0.40	0.02	0.49	−0.52
A	−0.90	−0.66	−0.22	−0.52	−0.52
M	−0.88	−0.97	−0.29	−0.15	−0.35
K	−0.81	−0.17	−0.31	−0.06	−0.52
V	−0.76	−0.64	−0.27	−0.05	−0.24
N	−0.69	0.23	−0.07	0.03	0.00
Y	−0.55	−0.30	0.05	–	−0.75
H	−0.46	−0.03	−0.16	0.33	−0.26
Q	−0.45	−0.21	−0.40	−0.30	−0.35
E	−0.40	−0.28	−0.35	−0.27	−0.32
R	−0.40	−0.20	−0.42	−0.08	−0.03
T	−0.29	−0.14	−0.08	0.27	−0.20
L	−0.26	−0.54	−0.39	−0.29	−0.35
G	−0.11	0.32	0.28	1.3	0.01
F	−0.08	−0.35	−0.49	0.02	−0.45
W	−0.03	−0.14	−0.64	–	−0.85
D	+0.05	0.42	0.07	0.52	0.20

[a] This work.
[b] Reference [20].
[c] Reference [14].
[d] Reference [19] converted from $\Delta\Delta G^{o}$ to ΔG^{o}.

secondary structure from protein sequence will require more than single helical propensities for each amino acid.

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