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Comparison of C−H···π and Hydrophobic Interactions in a β-Hairpin Peptide: Impact on Stability and Specificity

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Abstract: We have examined the impact of C−H···π and hydrophobic interactions in the diagonal position of a β-hairpin peptide through comparison of the interaction of Phe, Trp, or Cha (cyclohexylalanine) with Lys or Nle (norleucine). NMR studies, including NOESY and chemical shift perturbation studies, of the Lys side chain indicates that Lys interacts in a specific geometry with Phe or Trp through the polarized Cϵ. In contrast, Nle does not interact in a specific manner with the diagonal aromatic residue. Thermal denaturation provides additional support that Lys and Nle interact in fundamentally different manners. Folding of the peptide with a diagonal Trp···Lys interaction was found to be enthalpically driven, whereas the peptide with a diagonal Trp···Nle interaction displayed cold denaturation, as did the control peptide with a diagonal Cha···Nle interaction, indicating different driving forces for interaction of Lys and Nle with Trp. These findings have significant implications for specificity in protein folding and de novo protein design.

Introduction

Protein folding involves two critical elements, stability such that the adopted geometry is a favorable process and specificity to ensure that one particular geometry is favored among the many possible structures. A typical protein is stable only by about 5−10 kcal mol^{-1} over misfolded states. Hence, small differences in energy between a myriad of possible noncovalent interactions are summed up to provide a properly folded structure. The control of protein secondary and tertiary structure requires an understanding of how these weak forces provide both stabilization and specificity. It is generally accepted that the formation of a hydrophobic core is the energetic driving force for protein folding. However, the elements which constrain a protein to a single low-energy geometry are not as well defined.

Recent studies of cation−π interactions between aromatic and basic side chains in α-helices and β-hairpins have indicated that these interactions provide stability to peptide secondary structure by up to −0.5 kcal mol^{-1}.[1,5] In both secondary structures, significant interaction between the methylene adjacent to the ammonium group and the aromatic ring have been observed. This geometry of interaction is commonly observed in proteins[4,6] and has been proposed to be driven by either a cation−π interaction between the $CH_2(\delta+)$ and the π-cloud (CH···π interaction)[6] or alternatively by a hydrophobic interaction.[4]

The present study compares the interaction of the ϵ-methylene of a Lys side chain with an aromatic ring in a β-hairpin peptide to control peptides in which norleucine (Nle) or cyclohexylalanine (Cha) replace the Lys or Phe, such that only a hydrophobic interaction is possible. We expected that if the aryl···Lys interaction is hydrophobic in nature, it should behave similarly to the peptides containing an aryl···Nle or a Cha···Lys interaction. We utilized double mutant cycles to determine the interaction energies of residues in diagonal positions. We also obtained information on the geometry of the interaction through probing NOEs and side chain chemical shifts. Our results indicate that both hydrophobic and CH···π interactions provide modest stabilization of the hairpin through a diagonal interaction. Nonetheless, we find differences in the specificity of interaction between aromatic residues with Lys versus Nle with regard to geometry as well as enthalpic and entropic contributions to folding. These results indicate that the driving force for a C−H···π interaction between a polarized methylene, such as ϵ-methylene of Lys, and an aromatic residue is distinct from a classical hydrophobic effect and provides both stability and specificity to the peptide secondary structure.

Materials and Methods

Peptide Synthesis and Purification. The synthesis of all peptides was performed on an Applied Biosystems Pioneer peptide synthesizer using standard FMOC peptide synthesis methodology, where the reagents, synthesis conditions, and purification methods have been reported previously.[5] Once purified, peptides were lyophilized to powder and characterized by MALDI mass spectroscopy and NMR as described in the text.

NMR Spectroscopy. NMR samples were made to concentrations of 1−4 mM and analyzed on a Varian Inova 600 MHz instrument. Samples were dissolved in either 1:1 H_2O/D_2O or D_2O buffered to pH 4.2 (uncorrected) with 50 mM NaOAc-d_3, pH adjusted with HCl. 1D

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NMR spectra were collected using 32K data points and between 8 and 128 scans using a 1−3 s presaturation or solvent suppression. All 2D NMR experiments used pulse sequences from the Chempack software including TOCSY, DQCOSY, gCOSY, ROESY, and NOESY. 2D NMR scans were taken with 8−64 scans in the 1st dimension and 128−512 in the 2nd dimension. All spectra were analyzed using standard window functions (sinbell and Gaussian with shifting). Presaturation is used to suppress the water resonance. Mixing times of 100 or 200 ms were used for the NOESY and ROESY spectra. TOCSY spectra were recorded with 80 ms spin-lock. Assignments were made using standard methods as described by Wüthrich.[7] The temperature was calibrated using MeOH and ethylene glycol standards.

Results

***β*-Hairpin Design and Structure Determination.** The sequence investigated was based on a previously reported *β*-hairpin from our group[5] where X_1 is an aromatic or hydrophobic residue (Phe, Trp, and Cha) and X_2 is a cationic or linear aliphatic residue (Lys or Nle) (Figure 1). The overall charge of the peptides was +1 or +2 to promote water solubility and prevent aggregation. Concentration studies by NMR indicated that the peptides were monomeric under the conditions used in this study. An Asn-Gly sequence was incorporated at positions 6 and 7 as it has been shown to promote a type I′ turn.[8] Unless otherwise noted, all studies were conducted in D_2O with 50 mM acetate buffer, pH 4.2 (uncorrected).

To investigate the nature of the diagonal aryl···Lys interaction which we have reported previously,[5] we compared peptides **FK** and **WK** to the corresponding peptides in which the aromatic residue was replaced with cyclohexylalanine (Cha) and the cationic residue was replaced with norleucine (Nle) to give peptides **ChaK**, **FNle**, **WNle**, and **ChaNle**. These hydrophobic replacements allowed for investigation of the contribution of an electrostatic CH···*π* component to the interaction of the *ϵ*-methylene of Lys with the aromatic ring of Phe or Trp relative to an equivalent hydrophobic interaction.

To determine the structure of the investigated peptides, NOE assignments as well as α-hydrogen (Hα) and amide chemical shifts were examined. The disulfide-linked cyclic peptides and seven-residue truncated peptides in Figure 1 were used as control peptides to provide reference chemical shifts for the fully folded and random coil states, respectively.[9] Numerous nonadjacent NOEs were observed in the 12-residue and cyclic peptides throughout the length of the strands between residues that are separated by up to 12 residues in the primary sequence (Figure 2). This is consistent with a *β*-hairpin formation with the predicted strand register.

Qualitative analysis of the chemical shifts of the Hα and amide resonances is also consistent with *β*-hairpin structure. It has been shown that the formation of *β*-sheet structure results in a downfield shift of the Hα resonances. A change in chemical shift relative to the random coil state of greater than 0.1 ppm is considered significant.[10,11] The investigated peptides show many

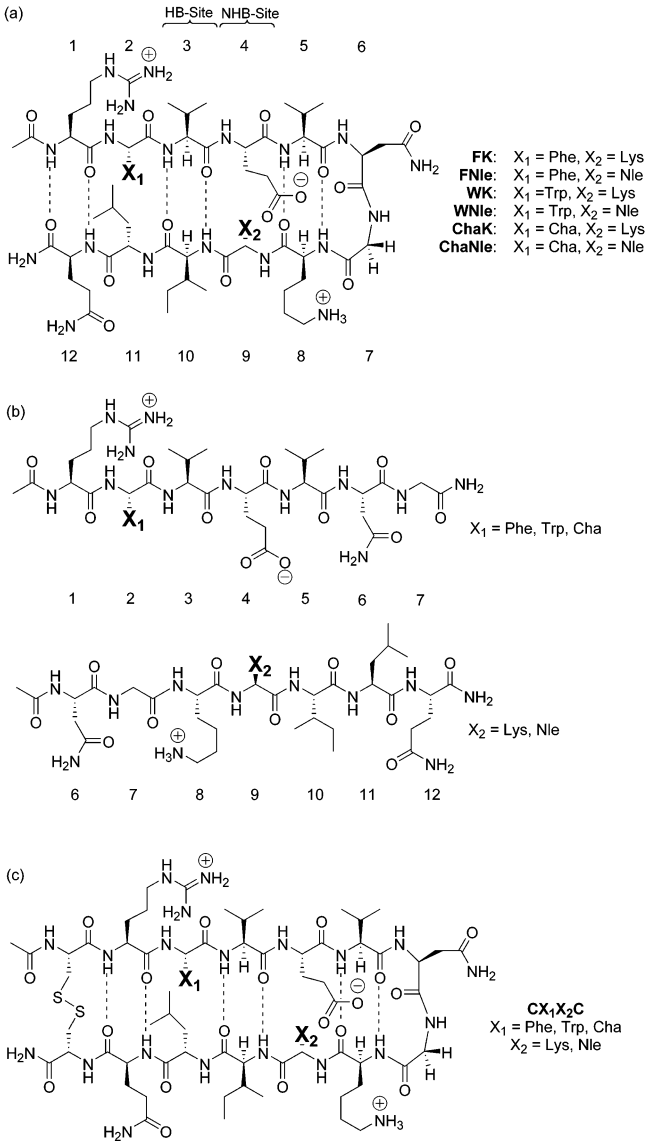

Figure 1. (a) Model peptide system (Ac-RX_1VEVNGOX_2ILQ-NH_2) used to probe the cation−*π* diagonal interaction. HB-site: hydrogen-bonded site. NHB-site: nonhydrogen bonded site. The diagonal interacting residues are in bold. Peptides are referred to by the diagonal residues in the text as $\mathbf{X_1X_2}$. (b) Seven-residue random coil control peptides. (c) Fourteen-residue cyclic control peptides. The cyclic peptides are referred to as $\mathbf{CX_1X_2C}$ in the text.

consecutive shifts greater than 0.1 ppm, indicating a well folded *β*-hairpin (Figure 3a). The cross-strand hydrogen bonding also results in the downfield shifting of the amide resonances. The greatest amount of shifting is experienced by the amide resonances in the hydrogen-bonded positions in the folded peptide. As can be seen in Figure 3b, residues Val3, Val5, and Ile10 are significantly downfield shifted in peptides **FK**, **WK**, and the cyclic peptide **CWKC**, up to 1.4 ppm for the cyclic peptide. This shifting indicates that those residues are in the hydrogen bonded sites of the *β*-hairpin, consistent with the structure shown in Figure 1. One would also expect Orn8 to be downfield shifted since it is in a hydrogen-bonded site, but the observed upfield shifting is likely due to its proximity to the *β*-turn.

The stabilities of the *β*-hairpins were determined by comparison of the Hα and Gly chemical shifts to random coil and

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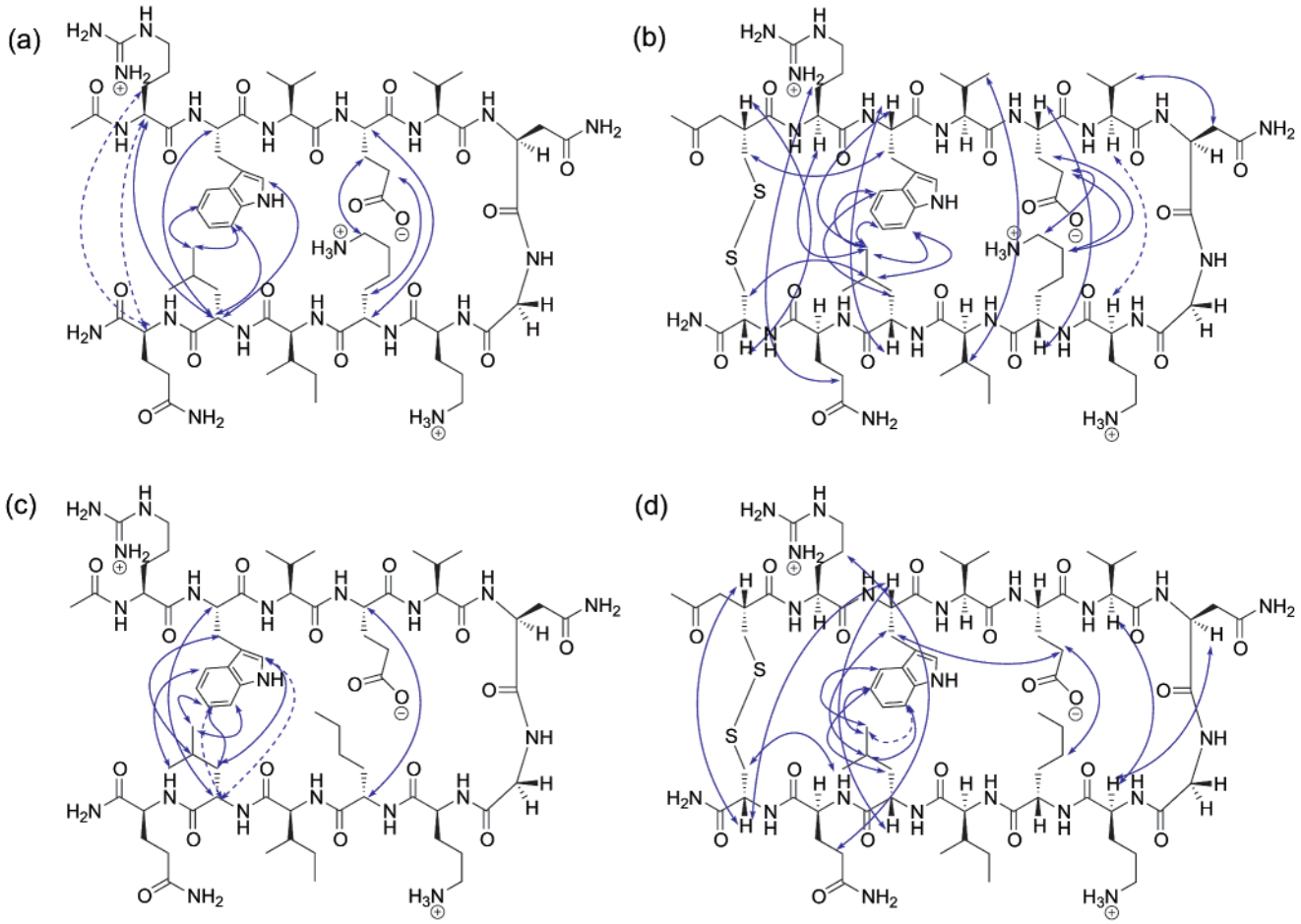

Figure 2. Long-range backbone and side chain cross-strand NOEs for peptides (a) **WK**, (b) **CWKC**, (c) **WNle**, and (d) **CWNleC**.

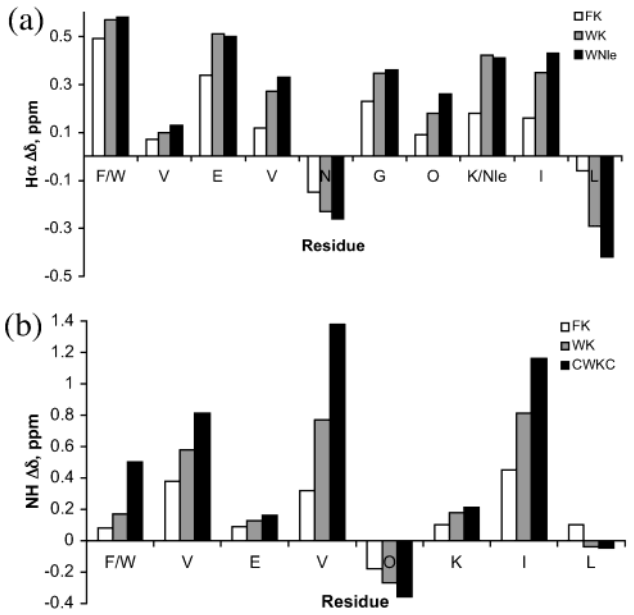

Figure 3. (a) Downfield shifting of α-hydrogens in **FK**, **WK**, and **WNle** relative to random coil. Conditions: D_2O, 50 mM acetate-d_3 buffer, pH 4.2, 298 K. (b) Downfield shifting of the amide hydrogens in **FK**, **WK**, and **CWKC** relative to random coil. Positions Val3, Val5, Orn8, and Ile10 are cross strand hydrogen bonded. The turn residues, Asn and Gly, are not shown.

fully folded reference states using eq 1, where δ_{100} is obtained from the cyclic control peptide and δ_0 is obtained from the seven-residue control peptides.[9]

$$\text{fraction folded} = (\delta_{\text{observed}} - \delta_0)/(\delta_{100} - \delta_0) \qquad (1)$$

The chemical shift difference ($\Delta\delta$) between the Hα and Hα′ Gly resonances in the turn has been shown to correlate well with Hα chemical shifts from strand residues in determining the extent of folding of β-hairpins.[12] Because the Gly Hα and Hα′ are farther upfield in the NMR spectrum than other Hα resonances, they provide a more accessible spectroscopic handle for investigation of β-hairpin stability, and hence, the extent of folding reported here is derived from the Gly Hα, Hα′ splitting. We have compared the Gly chemical shift difference in the peptides described here and confirmed that the hairpin stabilities determined in this way agreed with those from the Hα chemical shifts (see Supporting Information).

Characterization of the Diagonal Interaction between Residues 2 and 9. Magnitude of the Diagonal Interactions. Double mutant cycles have been used to measure noncovalent interactions in proteins,[13,14] peptides,[12,15,16] and organic intermolecular systems.[17,18] Figure 4 shows the double mutant

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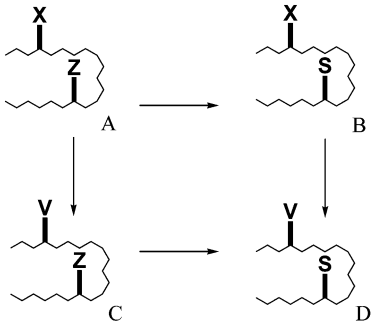

$$\Delta\Delta G_{XZ} = \Delta G_A - \Delta G_B - \Delta G_C + \Delta G_D$$

Figure 4. Schematic representation of the double mutant cycle used in this study. The double mutant cycle varies X and Z individually and jointly. The diagonal interaction between X and Z is determined by subtracting the stability of B and C from A and D.

Table 1. Stabilities of All Investigated Peptides

entry	peptide	$\Delta\delta$ (ppm)[a]	fraction folded[b]	ΔG° [c]
1	**FK**	0.230	0.51	−0.03
2	**FNle**	0.293	0.65	−0.38
3	**WK**	0.346	0.77	−0.72
4	**WNle**	0.383	0.86	−1.05
5	**ChaK**	0.224	0.53	−0.07
6	**ChaNle**	0.332	0.78	−0.76

[a] $\Delta\delta$ is the glycine H_α, $H_{\alpha'}$ chemical shift difference at 298 K. The error is ±0.005 ppm. [b] Fraction folded is determined from the Gly chemical shifts as described in the Materials and Methods section. Error is ±0.01 as determined from error in the temperature and chemical shift measurements. [c] ΔG° reflects the stabilities of the β-hairpins and not the diagonal interaction. The error is ±0.05 kcal mol^{-1}, as determined from error in the temperature and chemical shift measurements.

Table 2. Diagonal Interaction Energies (kcal mol^{-1}) at 298 K from Double Mutant Cycles[a]

	residue 9	
residue 2	K	Nle
F	−0.2	−0.1
W	−0.4	−0.2
Cha	−0.1	−0.3

[a] The error in the diagonal interaction energies is ±0.1 kcal mol^{-1} as determined from errors in temperature and chemical shift measurements (see Supporting Information).

cycle studied here. A single mutation of peptide A to give B or C disrupts the diagonal interaction of interest but may cause other changes to stability, such as a change in the β-sheet propensity. The double mutant, D, corrects for all changes other than the noncovalent interaction being investigated. Hence, the energy of the diagonal interaction can be isolated from other factors using the equation in Figure 4. The residues used in the mutants, Val2 and Ser9, are the same as those used in our previous study of cation−π interactions and were maintained here for consistency.[5] Table 2 displays the diagonal interaction energies determined from the double mutant cycle. There is no great difference between the magnitude of the cation−π and hydrophobic interactions; all are weakly stablilizing. Because the energetic values are nearly within error of one another, it is difficult to draw any conclusions from these values alone. Nonetheless, it appears that the interaction of Lys with an aromatic side chain is more favorable than the interaction with Cha, whereas Nle appears to interact more favorably with Cha than with the aromatic residues.

(18) Carver, F. J.; Hunter, C. A.; Livingstone, D. J.; McCabe, J. F.; Seward, E. M. *Chem. Eur. J.* **2002**, *8*, 2848−2859.

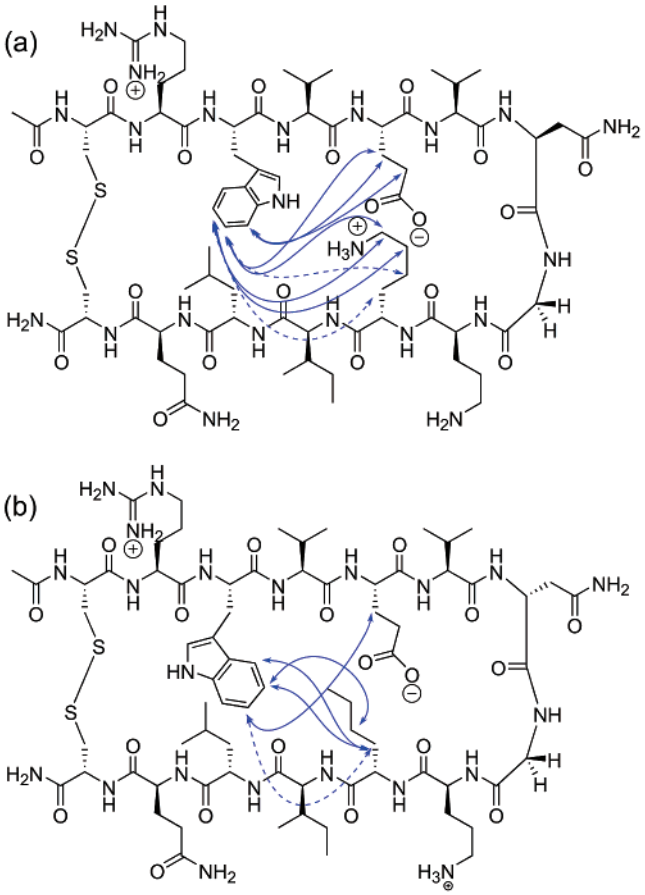

Figure 5. Lateral and diagonal NOEs for the cyclic peptides **CWKC** and **CWNleC**. The NOEs between lateral residues are separated from the other NOEs for clarity. The dashed lines represent weak NOEs. Conditions: D_2O, 50 mM sodium acetate-d_3 buffer, pH 4.2.

Diagonal NOEs. To characterize the nature of the diagonal interaction, we compared the NOEs on the NHB face of a peptide containing a diagonal Trp···Lys interaction to a peptide in which Lys was replaced with Nle. To avoid any disparities in NOEs arising from different hairpin populations (Table 1), we compared the side chain−side chain NOEs of the cyclic peptides, **CWKC**, and **CWNleC**, which are both fixed in a folded conformation (Figure 5). Diagonal NOEs are seen between residues X_1 and X_2 for both peptides **CWKC** and **CWNleC**. Nonetheless, there are differences in the NOEs between the side chains in these two peptides. There are more and stronger NOEs in **CWKC** than **CWNleC**. The diagonal NOEs between Trp and Lys in **CWKC** involve the δ- and ε-methylenes of the Lys side chain, near the polarizing ammonium group. In contrast, the diagonal NOEs between Trp and Nle in **CWNleC** involve the β- and γ-methylenes of Nle, suggesting a different geometric preference for Nle versus Lys.

Anisotropic Effects on Residue 9. The chemical shift perturbation of residue 9 arising from its proximity to an aromatic residue at position 2 provides information regarding the geometry and prevalence of the diagonal interaction. Thus, we compared the upfield shifting of Lys and Nle at position 9 when paired with Phe or Trp in position 2. As with the NOE studies, to account for any differences in the hairpin population, we also investigated the upfield shifting of residue 9 in the cyclic peptides, **CWXC**, where X is Lys or Nle.

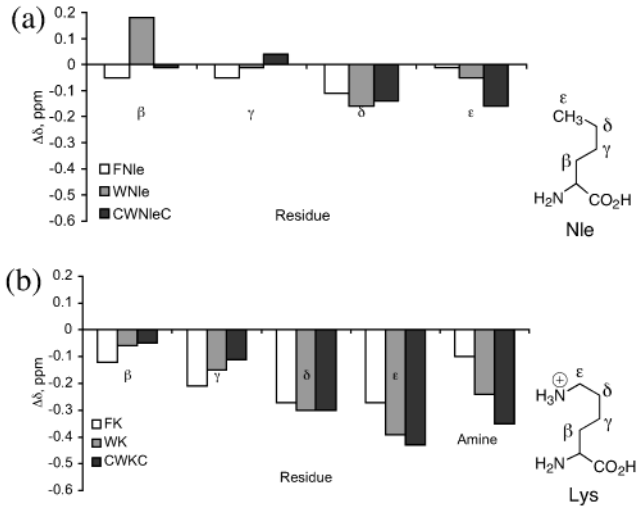

Figure 6. Upfield shifting of the diagonal side chains in position 9 relative to random coil values. (a) Nle side chain resonances relative to random coil chemical shifts in **FNle**, **WNle**, and **CWNleC**. (b) Lys side chain resonances relative to random coil chemical shifts in **FK**, **WK**, and **CWKC**.

In peptides **FNle**, **WNle**, and **CWNleC** only hydrophobic interactions are possible between the diagonal residues X_1 and X_2. The Nle side chain was included to assess the diagonal interaction uninfluenced by electrostatic interactions. In these three peptides, none of the Nle methylenes are significantly upfield shifted, the maximum being 0.16 ppm. The most shifted methylene is the δ-methylene followed by the ϵ-methylene (Figure 6a). This indicates that the δ-methylene is in closest proximity to the aromatic residue in position 2 but that the diagonal interaction is not a highly populated conformation of the Nle side chain.

Substituting Lys into the diagonal interaction at position 9 results in chemical shift changes that differ from that observed for Nle. While it is possible that Nle and Lys would interact with the aromatic residue in a similar manner through hydrophobic contacts between the alkyl chain and the aromatic ring, as Nle is structurally similar to Lys, the chemical shift differences implicate divergent methods of interaction. On the basis of the chemical shift perturbation of the Lys side chain, the most prominent site of interaction between Phe or Trp and Lys is between the ϵ-methylene and the aromatic ring (Figure 6b). This methylene is shifted by 0.28 ppm for **FK** and 0.39 ppm for **WK**. The ammonium group is shifted by only 0.1 and 0.24 ppm, respectively. The cyclic peptide demonstrates the same trends as **WK**. The greater magnitude of the anisotropic effect for Lys relative to Nle indicates that Lys spends more time in close proximity to Phe or Trp than does Nle, suggesting that the diagonal interaction is more favorable for Lys. Moreover, there is a difference in geometry, in that Cϵ, rather than Cδ, is most upfield shifted.

Thermal Denaturation Studies. The thermodynamic parameters of folding were determined through a nonlinear fitting of the fraction folded as determined from the Gly splitting[19] versus temperature (Table 3).[20] Peptides **WK** and **WNle** were chosen for the thermal denaturation studies as they were more folded than the corresponding peptides containing Phe. For comparison, we also investigated the thermal denaturation of **ChaNle**, in which only hydrophobic interactions are possible between the diagonal residues. The pH of the solution was also varied to investigate the contribution of the possible cross-strand salt bridge to folding.

The thermal denaturation of peptides **WK**, **WNle**, and **ChaNle** at pH 4.2 is shown in Figure 7a. The folding of all three peptides is enthalpically favorable and entropically

Table 3. Thermodynamic Parameters of **WK** and **WNle** at pH 4.2 and 1.5 as Determined from the Gly Splitting[a]

entry	peptide	pH	$\Delta H°$	$\Delta S°$	$\Delta C_p°$
1	**WK**	4.2	−3080 (30)	−7.8 (0.1)	−148 (4)
2	**WNle**	4.2	−1410 (70)	−1.2 (0.2)	−170 (9)
3	**ChaNle**	4.2	−1320 (20)	−1.8 (0.1)	−115 (2)
4	**WK**	1.5	−2980 (20)	−7.4 (0.1)	−170 (2)
5	**WNle**	1.5	−750 (90)	1.3 (0.3)	−210 (10)

[a] Units are as follows: $\Delta H°$, cal mol^{-1}; $\Delta S°$, cal mol^{-1} K^{-1}; and ΔCp°, cal mo^{-1} K^{-1}. All parameters are determined from the fitting to the van't Hoff equation as shown in Figure 7. The error is determined from the fitting. See Supporting Information.

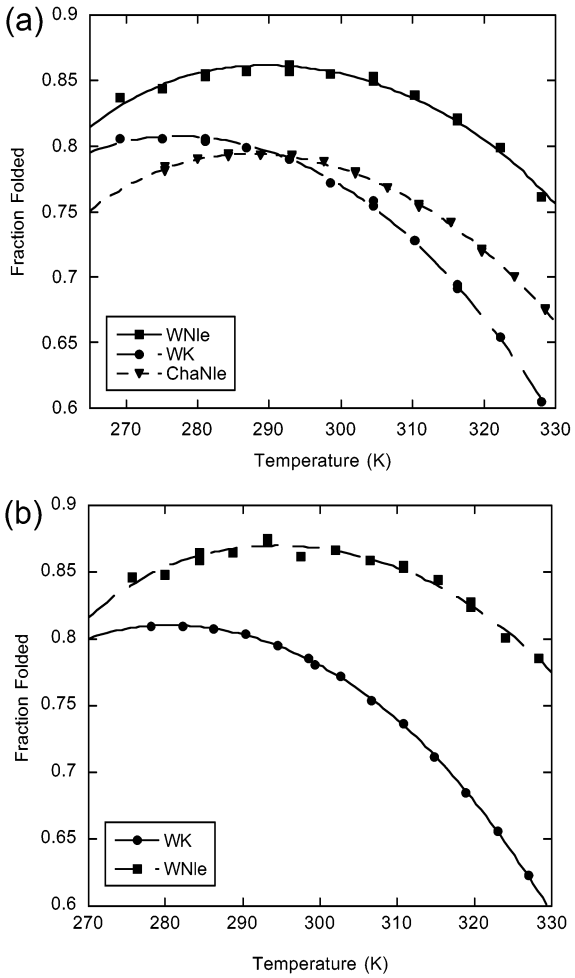

Figure 7. Thermal denaturation of **WK** and **WNle** at (a) pH 4.2 and (b) pH 1.5. The fraction folded was determined from the Gly splitting as described in the text. The curve represents the best fit to the data using the equation: fraction folded = $[\exp(x/RT)]/[1 + (\exp(x/RT)]$, where $x = [T(\Delta S°_{298} + \Delta C_p° \ln(T/298)) - (\Delta H°_{298} + \Delta C_p°(T - 298)]$. The temperature was calibrated with MeOH and ethylene glycol standards. See Supporting Information for error analysis.

(19) The thermodynamic parameters determined from Hα chemical shifts demonstrate the same trends as those determined from the Gly splitting. See Supporting Information.

(20) Maynard, A. J.; Sharman, G. J.; Searle, M. S. *J. Am. Chem. Soc.* **1998**, *120*, 1996−2007.

unfavorable at pH 4.2, yet the folding parameters differ for **WK** relative to those for **WNle** and **ChaNle**. Both **WNle** and **ChaNle** demonstrate cold denaturation, which is indicative of classical hydrophobic folding, whereas **WK** does not.[21] For peptide **WK** the enthalpic term is more favorable than it is for either **WNle** or **ChaNle** (Table 3, entries 1, 2, and 3). The entropic cost of folding of **WK** is concomitantly higher with a $\Delta S°$ of approximately -8 cal mol^{-1} K^{-1}, as compared to **WNle** and **ChaNle**, which have a smaller entropic cost of folding of about -1 to -2 cal mol^{-1} K^{-1} (Table 3, entries 2 and 3). Hence, although **WK** and **WNle** have similar stabilities, their thermodynamic profiles are divergent, whereas the thermodynamic parameters for **ChaNle** are nearly identical to those of **WNle** (Table 3, entries 2 and 3). This suggests that the nature of the interaction of Nle with Trp or Cha is similar and that the interaction of Trp and Lys is distinct.

To eliminate any contribution from a salt bridge between residues 4 and 9, the thermal denaturation was also performed at pH 1.5 (Figure 7b). As the pK_a of Glu is generally about 4.2−4.5, the side chain should be >99% protonated at this acidic pH. Nonetheless, the differences in $\Delta H°$ and $\Delta S°$ observed for **WK** at pH 1.5 differ by only about 5% (Table 3, entries 2 and 4). The difference in $\Delta C_p°$ is slightly larger. However, if $\Delta C_p°$ is set to the same value for **WNle** and **WK**, the other parameters are not greatly affected. Essentially there is very little observable difference between peptide **WK** at pH 1.5 and 4.2. The loss of the possible cross-strand salt bridge in **WK** does not significantly affect either the stability or the thermodynamic parameters of folding.

The effect of pH on **WNle** is somewhat greater (Table 3, entries 1 and 3). In particular, the folding of **WNle** is less enthalpically favorable and more entropically favorable at pH 1.5 than at pH 4.2. In essence, the peptide demonstrates a greater hydrophobic driving force at pH 1.5. This is likely because the protonated Glu is more hydrophobic in nature than glutamate and thus participates in hydrophobic interactions with other residues on the same face of the hairpin to a greater extent at pH 1.5.

Discussion

We have investigated the nature of the interaction between an aromatic residue and the ϵ-CH_2 of Lys by comparison to Cha and Nle as hydrophobic replacements for Phe or Trp and Lys, respectively. Double mutant cycles indicate that the diagonal interaction between aromatic residues and Lys or Nle was found to be energetically similar, providing modest stabilization to the β-hairpin. Nonetheless, it appears that the driving force for interaction differs, as determined by anisotropic effects, NOEs, and thermal denaturation.

The difference in upfield shifting of Nle and Lys indicates that a geometry in which the side chain of residue 9 is in close proximity to the aromatic ring is more highly populated for Lys than Nle. The limited amount of upfield shifting observed in the Nle peptides can be explained by the lack of a single low-energy conformation. NOE studies also indicate a difference in the interaction of Trp with Lys or Nle, suggesting that **WK** has a more tightly packed structure.

On the basis of upfield shifting of Lys, the favored geometries for peptides **WK** and **FK** direct the ϵ-methylene of Lys at the aromatic ring. This allows the protonated amine to remain solvated by water while packing the ϵ-methylene against the aromatic ring. Nonetheless, we do not believe that this represents a purely hydrophobic interaction, since the same interaction is not observed for Nle. The ϵ-CH_2 of Lys is highly polarized due to the neighboring ammonium group yet poorly solvated by water. Thus, it prefers to interact with an aromatic residue through a combination of electrostatic, hydrophobic, and van der Waals interactions, while the ammonium group remains exposed to solvent. The desolvation cost for this CH$\cdots\pi$ interaction is expected to be minimal, resulting in an enthalpically driven interaction.

Whereas the NOE and chemical shift data give a picture of the local interactions of the side chains, the thermal denaturation provides a global perspective. Thermal denaturation studies indicate that peptides **WK** and **WNle** have disparate folding determinants. Classically hydrophobically driven folding is evidenced by a weak or zero enthalpic term with weakly favorable entropy.[21] This describes the folding of the hydrophobic interactions observed in **WNle** and **ChaNle** quite well at pH 4.2 and even better at pH 1.5 for **WNle**. However, a single mutation from Nle to Lys results in measurable deviations from this folding behavior, such that folding of peptide **WK** is enthalpically more favorable and entropically less favorable than folding of **WNle** or **ChaNle**. This type of enthalpic folding profile has been observed in other peptide systems[22−25] and is frequently associated with tight packing of side chains.[22,24,26,27] Despite a number of nonpolar residues, the thermodynamic parameters for **WK** are not consistent with a dominant hydrophobic driving force. Cation$-\pi$ interactions are largely electrostatic in nature and are expected to be site specific and geometrically restrictive. The favorable enthalpy of folding for **WK** is consistent with an electrostatic interaction, and the less favorable entropic term suggests geometric restriction, consistent with a specific interaction, as indicated by the upfield shifting of the ϵ-methylene in Lys.

Significance of CH$\cdots\pi$ Interactions in Protein Structure. These studies reveal the applicability of CH$\cdots\pi$ interactions as a method for obtaining specificity in protein structure. Incorporation of Nle into the sequence appears to provide stability to the folded state by accessing a number of nonspecific interactions. In contrast, the diagonal interaction between an aromatic side chain and Lys or Arg provides stability to the β-hairpin through the formation of a specific interaction. This is reminiscent of the de novo designed coiled coils based on a Leucine zipper motif.[28] These designed proteins were found to take on a globular structure when Leu was the sole residue at the a and d sites, but lacked the single low-energy structure

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found in native proteins.[29−32] Incorporation of a buried polar interaction provided the specificity to give a nativelike structure, albeit at the cost of stability.[33,34] In the system reported here, a cation−π interaction between a polarized methylene and an aromatic ring provides specificity with no significant loss in stability relative to an equivalent hydrophobic interaction, thus providing specificity to folding.

An excellent example of the type of selectivity possible through the preferential interaction of a highly polarized methyl group with the face of an aromatic ring has been demonstrated recently in the crystal structure of the HP1 chromodomain bound to the histone H3 tail.[35] The histone tail only binds to the chromodomain when Lys 9 is methylated. The crystal structure indicates that the dimethyl Lys sits in an aromatic bowl made up of Tyr24, Tyr48, and Trp45, with a dissociation constant of 7 μM. In contrast, the unmethylated histone H3 tail does not bind to the chromodomain. The interaction has been proposed to consist of cation−π and van der Waals interactions, similar to the systems studied here.

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Conclusions

The combination of NOE data, thermodynamic parameters, and chemical shifts provide evidence that the CH···π interaction between Trp or Phe and the ϵ-methylene of Lys produces a specific interaction, which is manifested as a well-defined cluster of residues. These interactions differ from the features observed for **FNle** and **WNle**, in which the lack of a specific interaction is indicated by weak NOEs and chemical shift perturbations, as well as thermal denaturation studies, which provide a different energetic profile than for the peptides containing a CH···π interaction. These findings provide insight into the way weak polar interactions allow a protein to obtain the specificity necessary to form a single low energy folded state.

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Supporting Information Available: NMR assignments of all peptides, error analysis, and analysis of folding parameters from Hα chemical shifts. This material is available free of charge via the Internet at http://pubs.acs.org.

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