Investigation of the nature of the methionine–π interaction in β-hairpin peptide model systems

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Abstract

There are frequent contacts between aromatic rings and sulfur atoms in proteins. However, it is unclear to what degree this putative interaction is stabilizing and what the nature of the interaction is. We have investigated the aryl–sulfur interaction by placing a methionine residue diagonal to an aromatic ring on the same face of a β-hairpin, which places the methionine side chain in close proximity to the aryl side chain. The methionine (Met)–aryl interaction was compared with an equivalent hydrophobic and cation–π interaction in the context of the β-hairpin. The interaction between phenylalanine (Phe), tryptophan (Trp), or cyclohexylalanine (Cha) and Met stabilized the β-hairpin by −0.3 to −0.5 kcal mole^{-1}, as determined by double-mutant cycles. The peptides were subjected to thermal denaturations that suggest a hydrophobic driving force for the interactions between Met and Trp or Cha. The observed interaction of Met or norleucine (Nle) with Trp or Cha are quite similar, implying a hydrophobic driving force for the Met–π interaction. However, the thermodynamic data suggest that there may be some differences between the interaction of Met with Trp and Phe and that there may be a small thermodynamic component to the Met···Phe interaction.

Keywords: β-hairpin peptide; peptide secondary structure; pairwise interactions; noncovalent interactions; sulfur-aromatic interactions; NMR

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The aryl–sulfur interaction has been proposed to be a favorable interaction because of the observed proximity of cysteine (Cys) and methionine (Met) to aromatic side chains in protein crystal structures (Reid et al. 1985; Pal and Chakrabarti 1998, 2001; Samanta et al. 2000) and small molecule crystal structures (Zauhar et al. 2000). Analysis of the Protein Data Bank reveals that tryptophan (Trp) is the most overrepresented residue near disulfide bonds (Petersen et al. 1999). The observed geometry of the interaction places one of the sulfur atoms of the disulfide bond and the adjacent CH_2 of Cys in close proximity to the aromatic ring. However, in some cases it appears that there may be some hydrogen bonding character in an S···H–Ar interaction. Although the contacts between aromatic rings and sulfur groups are numerous, there are no energetic data from these structural investigations and it is not clear what drives these interactions. The favorable interactions may arise from hydrogen bonding to the aryl hydrogens, SH–π interactions, electrostatic interactions, or hydrophobic interactions.

Investigations in model α-helix systems have shown that there is a stabilizing interaction between Met or Cys and phenylalanine (Phe). Both a 15-and 17-residue helix show that the interaction of Met with Phe provides up to −0.75 kcal mole^{-1} and the Phe–Cys interaction has been estimated to provide up to −2 kcal mole^{-1} (Stapley et al. 1995; Viguera and Serrano 1995). In these systems, the strongest NOEs are observed between the aromatic Phe protons and the Met ε-CH_3 group. Although there was significant stabilization observed, the nature of the interaction was not addressed. A flavin receptor complex has also shown that facial contact between the aromatic rings of flavin and a sulfur enhance binding by ~1 kcal mole^{-1} in CH_2Cl_2 (Breinlinger et al. 1998). Computation studies of this system indicate that the nature of the stabilization is electrostatic, as there is little distortion of the sulfur electron cloud (Rotello 1998).

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Site-directed mutagenesis of proteins has offered conflicting results concerning the nature of the aryl–sulfur interaction. The substitution of Met 32 in staphylococcal nuclease with either leucine (Leu) or isoleucine (Ile) was computationally predicted to increase the stability of the protein by as much as 1.6 kcal mole^{-1}. However, both of these mutations were found to be destabilizing by 0.6 and 0.8 kcal mole^{-1}, respectively (Spencer and Stites 1996). This is in contrast to the findings of the binding site of herpes simplex virus type 1 thymidine kinase. Met 128 is in facial contact with thymidine, but computational studies indicate that there are no changes in the polarization of the sulfur nor charge transfer between the Met and thymidine (Alber et al. 1998). Alber et al. propose that Met 128 interacts via hydrophobic and dispersion interactions. This is supported by the fact that substitution with other hydrophobic residues is possible with retention of activity (Pilger et al. 1999). Theoretical studies of model systems that have attempted to dissect the energetic contributions to the aryl–sulfur interaction do not provide a unified picture of the interaction. Computational models between methanethiol and benzene suggest that the most favorable geometry places the methyl group above the plane of the aromatic ring with the S-H bond pointed away from the ring (Cheney et al. 1989). The direct interaction with the polarizable methyl group seems to be more favorable than an SH–π interaction. The stabilization gained was calculated to be −3 kcal mole^{-1}.

The frequent proximity of an aromatic ring and a sulfur atom implies a favorable interaction, but the geometry, magnitude, and nature of this interaction is not well understood. The methyl and methylene atoms flanking the sulfur of Met have been implicated, as well as sulfur atom itself. It is also unclear whether the face or edge of the aromatic ring is the preferred region for interaction. We sought to use a β-hairpin peptide that is predisposed to place Met and an aromatic residue in close proximity to explore the nature and geometry of the putative aryl–sulfur interaction. Phe and Trp were investigated to determine if the electron density on the face of the ring played a role. The interaction of the aromatic ring with Met was compared with the interaction with lysine (Lys) or norleucine (Nle), which provide representative electrostatic and hydrophobic interactions with the aromatic residue. From these studies, we find that at ambient temperature, the sulfur–aryl interaction demonstrates features consistent with a primarily hydrophobic driving force.

Results

Peptide design

The model system used to investigate the nature of an aryl–sulfur interaction was based on a β-hairpin peptide previously used in our lab to study diagonal cation–π interactions and side-chain specificity (Fig. 1; Tatko and Waters 2003, 2004). The overall charge of the peptides was +1 to +2 to increase solubility and prevent aggregation. An Asn–Gly turn was incorporated, as it has been shown to promote a type I′ turn (Griffiths-Jones et al. 1998). The cross-strand hydrogen bonds that contribute to the stabilization of the hairpin occur between positions 3 and 10 and 5 and 8. This places the X_1 and X_2 residues diagonal from one another in the more flexible nonhydrogen-bonded (NHB) sites. Aromatic residues Phe and Trp were incorporated at position X_1 and Met was incorporated at position X_2. In addition, several control sequences were investigated. Cyclohexylalanine (Cha) was incorporated at X_1 as a hydrophobic replacement for Phe, and Nle was incorporated as a hydrophobic control for Met at X_2. Lastly, we also incorporated Lys at X_2 as an electrostatic control for Met, because we have shown previously that Lys interacts with Phe or Trp via a favorable cation–π interaction in this model system. In addition, we studied the cyclic peptides CWMC, CWKC, and CWNleC and seven-residue peptides as controls representing the fully folded and random coil states (see Materials and Methods).

The aryl–sulfur interaction has been proposed to have a number of different potential geometries and energetic components. In order to accurately probe the sulfur–aromatic interaction, there must be proximity as well as inherent flexibility in the model system. Previous studies have indicated that when an aromatic ring is diagonal from a Nle, the δ position of Nle side chain interacts with the aromatic ring to the greatest extent (Tatko and Waters 2004). However, when a Lys is diagonal from an aromatic ring, the ε-position has the greatest interaction, implying some conformational flexibility. This system allows the diagonal residues to access the most favorable orientation, which may include Ar-H···S interactions, sulfur–π interactions, or hydrophobic interactions involving the γ-CH_2 and ε-CH_3 adjacent to the sulfur atom.

Characterization of β-hairpin structure and side-chain···side-chain interactions

The parent sequence has been reported previously and has been shown to take on a β-hairpin conformation that tolerates a wide range of substitutions (Tatko and Waters 2003, 2004). Characterization via long-distance NOE analysis of the new substitutions reported here indicates that no major change in structure is induced by these residues (not shown; Tatko and Waters 2003, 2004). Investigation of the Hα and amide chemical shifts also provided evidence that these peptides were well folded (vide infra).

Hα chemical shifts

The adoption of a β-hairpin structure causes shifting to the Hα and amide resonances. Downfield shifting of the Hα

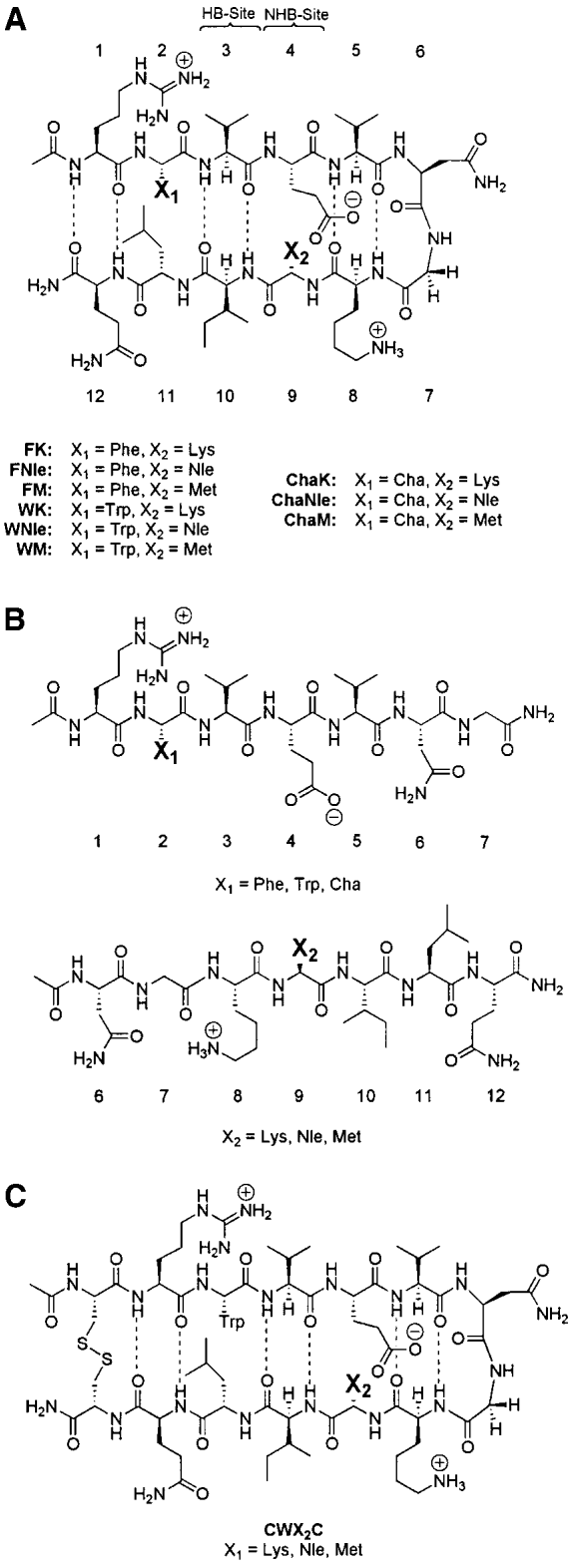


Figure 1. (*A*) The 12-amino acid model system (Ac-RX$_1$VEVNGOX$_2$ ILQ-NH$_2$). The diagonal interacting residues are in bold. Peptides are referred to by the diagonal residues, X$_1$X$_2$, in the text. (*B*) The seven-residue peptides used as random coil controls. (*C*) The disulfide bonded peptides CWX$_2$C used as fully folded controls.

protons, relative to random coil, has been shown to correlate with increasing β-hairpin conformation. The investigated peptides demonstrate significant downfield shifting of multiple adjacent residues, with the exception of the terminal residues, which are frayed (Fig. 2). Chemical shifts as large as 0.62 ppm are observed where a downfield shift of 0.1 ppm is considered significant, indicating that these β-hairpins are well folded (Wishart et al. 1992).

Amide chemical shifts

Change in the chemical shift of the amide resonances results from cross-strand hydrogen bonding. The resulting downfield shift indicates the extent of folding as well as the alignment of the individual strands. As can be seen in Figure 3, there is significant shifting of the amide resonances. The magnitude of shifting is particularly large at the 3, 5, 8, and 10 positions, consistent with the hydrogen-bonding pattern in the structure in Figure 1. The upfield shift of Orn 8 results from the turn geometry. The amount of amide chemical shifting is greatest for WM, up to 1.2 ppm, and least for VM, which is indicative of the stabilities of the peptides and correlates with the observed Hα chemical shifts.

The stability of the peptides was determined by comparison of the NMR chemical shifts of the α-protons and the diastereotopic glycine protons (Griffiths-Jones et al. 1999) of the peptide of interest relative to those of reference compounds representing the random coil and fully folded states by using equation 1, as described in Materials and Methods. The observed stabilities of the peptides indicate that they are well folded (Table 1). All of the peptides containing diagonal interactions are >50% folded, and the peptides range in stability by ~1 kcal mole^{-1}.

Magnitude of the diagonal interaction

To determine the magnitude of the diagonal interaction, we performed a double-mutant cycle (Fig. 4). In the double-

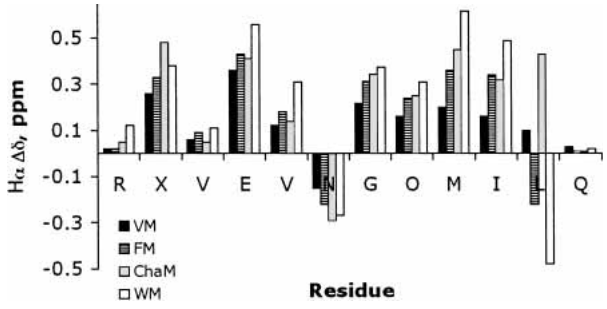


Figure 2. The difference in β-hairpin and random coil chemical shifts. Asn is shifted upfield as is expected for the turn residue. The Gly Hα is the difference in chemical shift of the diastereotopic Hαs. The upfield shifts of Leu in FM and WM are due to the anisotropic effects. Residues Arg 1 and Gln 12 are frayed and show little shifting from random coil values.

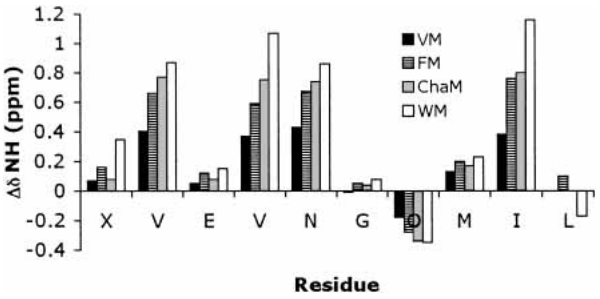
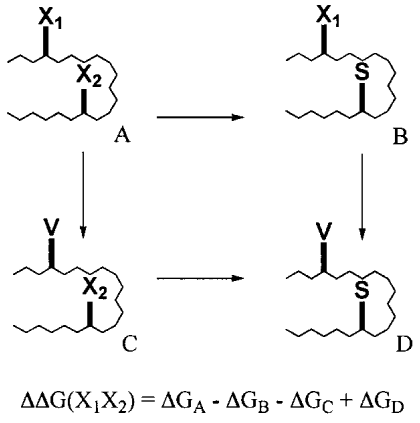


Figure 3. The downfield chemical shift of the amide resonances relative to random coil. Positions Val 3, Val 5, Orn 8, and Ile 10 are cross-strand hydrogen bonded. The upfield shift of the Orn 8 is caused by the turn geometry.

mutant cycle, both of the interacting residues, X_1 and X_2, are mutated individually and together. The individual mutants, B and C, disrupt the side-chain–side-chain interaction in A, but may result in other changes that affect the stability of the β-hairpin, such as the β-sheet propensity. The double mutant, D, corrects for all unintended changes that affect the β-hairpin stability. Thus, the sum of the stabilities of peptides A and D minus those of the single mutants, B and C, provides the side-chain–side-chain interaction of X_1 and X_2. In the system investigated here, X_1 was mutated to Val and X_2 was mutated to Ser, as in previous studies of diagonal interactions.

All of the observed diagonal interactions are stabilizing in the range of −0.1 to −0.5 kcal mole^{-1} (Table 2). The diagonal interaction of the Met residue is greatest with Cha at −0.5 kcal mole^{-1}. In contrast, the interaction of Cha with Lys is only −0.1 kcal mole^{-1}. The fact that Met interacts to the greatest extent with the aliphatic Cha suggests that Met interacts more favorably via hydrophobic packing. The interaction energy of Met with Phe or Trp is similar to that of Lys or Nle with Phe or Trp, thus providing little insight into the nature of the Met···aromatic interaction.



$$\Delta\Delta G(X_1X_2) = \Delta G_A - \Delta G_B - \Delta G_C + \Delta G_D$$

Figure 4. The double-mutant cycle varies X_1 and X_2 individually and jointly. The diagonal interaction energy between X_1 and X_2 is determined by subtracting the stability of *B* and *C* from *A* and *D*.

Anisotropic effects on residue 9

Proximity of a proton to the π-cloud of an aromatic ring results in upfield shifting of the proton resonance in the NMR spectrum. Thus, the chemical shifts of the Met side chain provide a probe of the position in the Met side-chain that interacts most favorably with the aromatic ring. Because the sulfur does not yield an observable signal, the proximity of it to the aromatic ring must be inferred from the adjacent γ-CH_2 and ε-CH_3 resonances. We investigated the upfield shifting of the Met side chain in FM, WM, and CWMC and compared the results to the upfield shifting of Lys and Nle when paired with Phe and Trp. The inclusion of a fully folded reference peptide provides an estimate of the maximum attainable upfield shifting because all of the cyclic peptides are equally folded.

The chemical shift differences observed for the aryl–Met interaction are very modest (Fig. 5). The greatest perturbation occurs in the WM and CWMC peptides at the ε-CH_3 position but is only 0.17 ppm. Most of the chemical shift changes are <0.1 ppm. The upfield shifting of the Met and Nle side chains is very similar. Because Nle has no functionality on the side chain, it is only capable of interacting in a hydrophobic manner. In contrast, the highly polarized Lys side chain is upfield shifted to a greater extent, with a maximum at 0.43 ppm, which has been shown to result from an attractive cation–π interaction with Phe or Trp (Tatko and Waters 2003). Thus, the upfield shifting data suggest that Met interacts with Phe or Trp similarly to Nle. If there was a significant site-specific interaction along the Met side chain, a greater chemical shift difference would be anticipated, as has been observed for peptide WK (Tatko and Waters 2003).

Thermal denaturation studies

To further investigate the driving force of the aryl–sulfur interaction, we performed thermal denaturations and fit them with the van't Hoff equation. The Trp series provides a good comparison, as all of the peptides are of similar stabilities. Comparison of WNle and WK provides a framework to understand the aryl–Met interaction. WK has previously been shown to have a diagonal cation–π interaction that impacts the folding of the peptide (Tatko and Waters 2003). Because a cation–π interaction is largely electrostatic in nature, it results in an enthalpic stabilization of folding of >3 kcal mole^{-1} and an entropic cost of ~8 cal mole^{-1} K^{-1} (Table 3). In contrast, the Trp and Nle residues in peptide WNle can interact only through hydrophobic packing, which results in peptide folding that displays a weaker, favorable enthalpic term of −1.4 kcal mole^{-1}, with a mildly unfavorable entropic term of −1.2 cal mole^{-1} K^{-1},

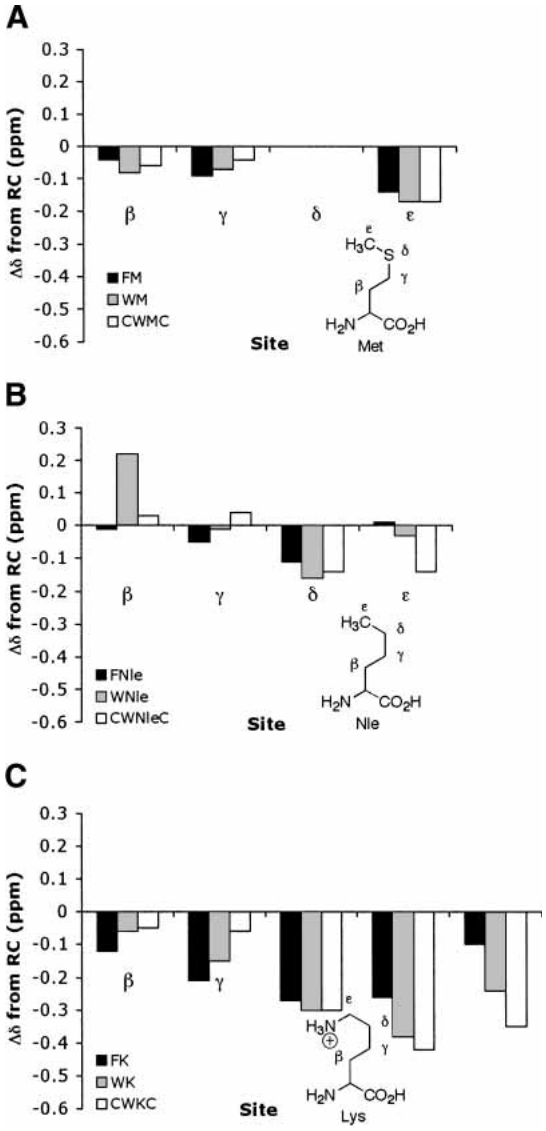


Figure 5. The upfield shifting of the side chains in position 9 relative to random coil values. (*A*) Met side-chain resonances relative to random coil chemical shifts in FM, WM, and CWMC. (*B*) Nle side-chain resonances relative to random coil chemical shifts in FNle, WNle, and CWNleC. (*C*) Lys side-chain resonances relative to random coil chemical shifts in FK, WK, and CWKC.

and exhibits cold denaturation (Fig. 6A; Tatko and Waters 2004). Peptide WM also shows a considerable amount of cold denaturation, and the thermodynamic parameters of folding reveal a weakly favorable enthalpic term of less than –0.7 kcal mole^{-1}, with a weakly favorable entropic term of ~+1 cal mole^{-1} K^{-1}. The parameters demonstrated by WM are similar to those of WNle and are consistent with a hydrophobic driving force.

Replacing the aromatic residue with Cha eliminates possible electrostatic interactions between the aromatic ring and the sulfur atom of Met or the polarized Lys. Consequently, the only possible interaction between Cha and Met, Lys, or Nle is hydrophobic. If Met is capable of interacting primarily in an electrostatic manner, then differences may manifest themselves between the Nle and Met residues. In fact, the thermal denaturation of ChaM and ChaNle are almost identical in the degree of folding and the effect of temperature. Both exhibit cold denaturation and have parameters of folding that are within error of one another: ΔH° of –1.5 kcal mole^{-1} and ΔS° of –2.5 cal mole^{-1} K^{-1}. The difference in the ΔCp° term is slightly greater than error, but the values are similar. The ChaK peptide shows similar weak enthalpic gain to ChaM and ChaNle, but the entropic cost is greater at –5.6 cal mole^{-1} K^{-1}. However, it is important to note that the large difference in the fraction folded between ChaK and ChaM or ChaNle may mitigate the comparison of the enthalpic and entropic terms because the global stabilities are so different.

When paired with Phe instead of Trp, Met and Nle are less similar. Neither demonstrates significant cold denaturation, and folding of FM is more enthalpically favorable and less entropically favorable than FNle, in contrast to the case

Table 1. *Stabilities of the Met, Lys, and Nle peptides*

Peptide	Δδ[a]	Fraction folded[b]	ΔG°[c]
FK	0.230	0.51	–0.03
WK	0.346	0.77	–0.72
ChaK	0.224	0.52	–0.05
FM	0.313	0.70	–0.50
WM	0.376	0.84	–0.97
ChaM	0.344	0.80	–0.82
FNle	0.293	0.65	–0.38
WNle	0.383	0.86	–1.05
ChaNle	0.332	0.77	–0.72

[a] Δδ is the glycine Hα chemical shift difference at 298K. Error is ± 0.005 ppm.
[b] Fraction folded is determined from the Gly chemical shifts as described in Materials and Methods.
[c] The ΔG reflects the stabilities of the β-hairpins and not the diagonal interaction.

Table 2. *Magnitude of the diagonal interactions as determined from double-mutant cycles*

Peptide	Diagonal interaction[a]
FK	–0.2
WK	–0.4
ChaK	–0.1
FM	–0.3
WM	–0.3
ChaM	–0.5
FNle	–0.1
WNle	–0.3
ChaNle	–0.4

[a] The error is ±0.1 kcal mole^{-1}.

when Met and Nle are paired with Trp. This may indicate a preference for a different type of interaction, such as a C-H···S interaction instead of a sulfur···π interaction (Breinlinger et al. 1998; Rotello 1998), but we are unable to differentiate between the possible interaction geometries in this system.

Discussion

Magnitude of Aryl–Met interactions

Through a double-mutant cycle, we have found that both Phe and Cha interact more strongly with Met than with Lys or Nle (Table 2), whereas the diagonal interaction between Trp and Nle or Met is similar in magnitude. The aromatic rings Phe and Trp can interact in a variety of ways, including through van der Waals, electrostatic, and hydrophobic interactions. Cha, however, can only interact through hydrophobic interactions. The significant –0.5 kcal mole^{-1} diagonal interaction between Cha and Met suggests that Met is capable of a sizable hydrophobic interaction.

The observed interaction between Phe and Met is stabilizing by –0.31 kcal mole^{-1}. This is in good agreement with previous investigations in α-helices that found a stabilization between Phe and Met between –0.2 and –0.75 kcal mole^{-1}, depending on which residue was in the *i* and *i + 4* position (Viguera and Serrano 1995). Because these different orientations in the α-helix gave different stabilities, the small variance between α-helices and β-hairpins are likely due to side-chain orientation and differences in conformational restriction. The magnitude of interaction of Phe or Trp with Met is greater than or equal to the interaction with Nle. This is consistent with mutation studies that have shown greater stabilities with Met over Leu or Ile in staphylococcal nuclease (Spencer and Stites 1996).

Geometry from Met chemical shifts

The extent of upfield shifting of the Met side chain was compared with Nle and Lys to determine differences in

Table 3. *Thermodynamic parameters of folding*

Peptide	ΔH°	ΔS°	$\Delta C_p°$
WM	–670 (50)	1.1 (0.2)	–204 (7)
WNle	–1410 (70)	–1.2 (0.2)	–170 (9)
WK	–3080 (30)	–7.9 (0.1)	–149 (4)
ChaM	–1560 (30)	–2.5 (0.1)	–177 (5)
ChaNle	–1520 (20)	–2.5 (0.1)	–144 (2)
ChaK	–1740 (20)	–5.6 (0.1)	–109 (3)
FM	–2260 (30)	–5.9 (0.1)	–142 (5)
FNle	–1610 (20)	–4.1 (0.1)	–117 (2)
FK	–2310 (20)	–7.6 (0.1)	–106 (2)

Units are as follows: ΔH°, cal mole^{-1}; ΔS°, cal mole^{-1} K^{-1}; and $\Delta C_p°$, cal mole^{-1} K^{-1}. All parameters are determined from the fitting to the van't Hoff equation, as shown in Fig. 6. The error is determined from the fitting.

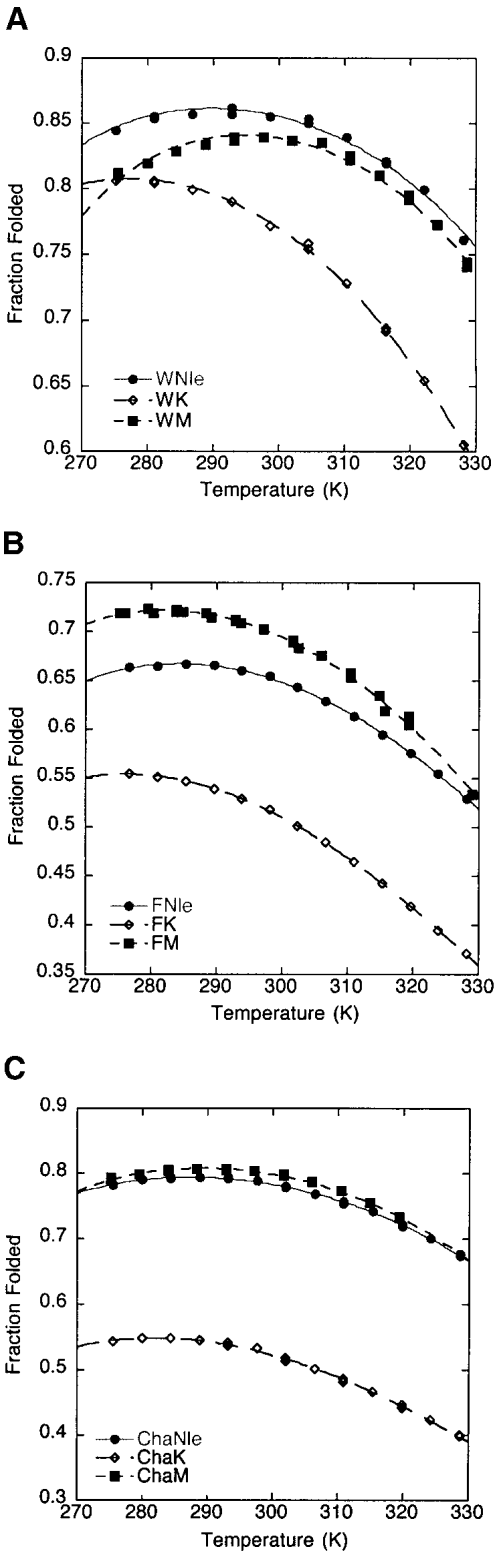


Figure 6. Thermal denaturation plots of (*A*) WK, WNle, and WM; (*B*) FK, FNle, and FM; and (*C*) ChaK, ChaNle, and ChaM. The fraction folded is determined from reference compounds. The fitting of the curve was accomplished by using the following equation: fraction folded = (exp[X / RT]) / (1 + exp[x / RT]), where $x = \{T(\Delta S°_{298} + \Delta C_p° \ln[T / 298]) - (\Delta H°_{298} + \Delta C_p° [T - 298])\}$.

proximity to the aromatic residue and the nature of the interaction. The cation–π interaction restrains the side-chain geometry in a specific orientation, which results in the large upfield shifting of the side-chain resonances (Tatko and Waters 2003, 2004). The upfield shifting observed for Nle is quite small because only nonspecific hydrophobic interactions are possible. The comparison of the Met side chain to Nle and Lys clearly demonstrates a similar pattern to Nle. Because the sulfur atom is not observed, the direct interaction between an aromatic ring and the sulfur atom cannot be assessed. However, if the interaction between an aromatic ring and a sulfur atom were specific, then one would expect that the adjacent protons would be significantly shifted, as seen with Lys, particularly when paired with Trp, which has a larger facial surface area. The incorporation of the cyclic CWMC demonstrates that even with a restrained backbone, the resonances of the Met side chain are not significantly upfield shifted. These results are consistent with a nonspecific interaction between the Met side chain and an aryl ring.

Nature of the Aryl–Met interaction

The thermodynamic parameters of folding for WM suggest a significant hydrophobic component to the aryl–sulfur interaction. Both ChaM and WM exhibit cold denaturation, which is a hallmark of a hydrophobic driving force. It follows that the Trp···Met interaction is principally hydrophobic in nature. It is possible that the observed cold denaturation arises from the greater conformational freedom of Met relative to Lys or Nle (Gellman 1991), rather than differences in the nature of the interactions. However, the fact that the thermal denaturation of ChaNle and WNle are nearly identical argues against this possibility. Hence, the hydrophobic nature of the Trp···Met interaction is likely because, although sulfur is quite polarizable, it is not very electronegative. In fact, its electronegativity is the same as that of carbon. Hence, the adjacent methyl and methylene groups are not significantly polarized relative to the methylene of Lys, such that Met is more similar to Nle than to Lys. It is not clear at this time why the thermodynamic profile for FM differs from WM, but it may be the result of a different interaction geometry, such as an Ar-H···S interaction, which has a greater electrostatic or dispersion component.

The apparent hydrophobic driving force for WM is in good correlation with a density functional investigation into the binding of thymidine to herpes simplex virus type 1 thymidine kinase (Alber et al. 1998). In this system, it was observed that the Met in the binding pocket was only involved in steric and hydrophobic interactions with no observed perturbation of the HOMO or LUMO, which would have indicated an electrostatic interaction.

It is interesting to note that peptides WM and WNle are among the best-folded β-hairpin peptides reported to date, and yet the only two well-folded peptides that exhibit cold denaturation and an entropic driving force for folding. This is consistent with the fact that most other well-folded β-hairpins ($\Delta G^\circ < -1$ kcal mole^{-1}) consist of an aromatic cluster, and aromatic interactions have been shown to be enthalpically driven.

Conclusion

A well-folded β-hairpin that positions the sulfur atom of Met in close proximity to the aromatic ring of Phe or Trp has been investigated. It appears from the magnitude of the double-mutant cycle that there is an attractive interaction between the residues, but limited chemical shifts of the Met side chain and the thermodynamic parameters of folding implicate classical hydrophobic interactions as the major contributor. Although the thermodynamic parameters of folding for FM suggest there may be increased electrostatic or dispersion interaction, further investigation is required to fully understand the aryl–sulfur interaction. We are continuing to investigate the influence of the electrostatic potential of the aromatic ring on this interaction with unnatural residues.

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 (Tatko and Waters 2003, 2004). 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 d_3-NaOAc, pH adjusted with HCl. 1D NMR spectra were collected using 32K data points and between 8 and 128 scans using a 1- to 3-sec 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 first dimension and 128–512 in the second 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 msec were used for the NOESY and ROESY spectra. TOCSY spectra were recorded with 80-msec spin-lock. Assignments were made using standard methods, as described by Wüthrich (1986). The temperature was calibrated using MeOH and ethylene glycol standards.

Peptide stability

The stability of the peptides was determined by comparison of the NMR chemical shifts of the α-protons and the diastereotopic glycine protons (Griffiths-Jones et al. 1999) of the peptide of interest relative to those of reference compounds representing the random coil and fully folded states by using equation 1 for Hα chemical shifts and equation 2 for Gly splitting. For each β-hairpin, two seven-residue peptides (residues 1–7 and 6–12), representing each strand of the β-hairpin, were synthesized and characterized as random coil reference states. The fully folded state was attained by forming the cyclic disulfide-bonded 14-residue peptide with a Cys appended to the N and C termini. We confirmed that the cyclic peptides adopt a β-hairpin conformation through characterization of the amide chemical shifts and cross-strand NOEs. The disulfide is in the NHB site, which has been shown to be preferred over the HB site. The formation of the disulfide bond is affected by air oxidation under high dilution conditions in MeOH. Chemical shifts of the fully folded and random coil states were taken at 298K because negligible differences in chemical shift were found between 273K and 298K (<0.01 ppm).

$$\text{Fraction Folded (H}\alpha) = \frac{(\delta_{\text{observed}} - \delta_{\text{random coil}})}{(\delta_{\text{fully folded}} - \delta_{\text{random coil}})}$$

$$\text{Fraction Folded (Gly)} = \frac{\Delta\delta\text{Gly}_{\text{obs}}}{\Delta\delta\text{Gly}_{100}}$$

In determining the fraction folded, there is typically good agreement between values determined by the Hα and the Gly chemical shift for peptides with Asn–Gly turns (Griffiths-Jones et al. 1999; Tatko and Waters 2002). Previous investigations have indicated that the α-hydrogens in H-bonded sites of the β-hairpin provide the best assessment of the fraction folded because those sites are less flexible than are the NHB sites. However, in the system studied here, the α-hydrogens in HB sites are perturbed to different extents by the proximity of the aromatic residues, resulting in inconsistent determination of the fraction folded from these residues. In contrast, the glycine Hα, Hα' chemical shift difference in not perturbed by the aromatic substitutions. Hence, the glycine chemical shift difference was the primary method used to determine the extent of folding, but the agreement between Hα and glycine was found to be good when not affected by other factors.

Electronic supplemental material

NMR assignments of all peptides are provided.

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