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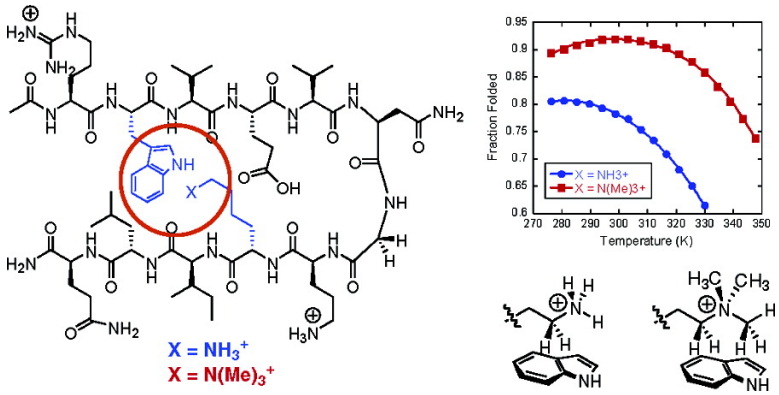

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Influence of *N*-Methylation on a Cation−π Interaction Produces a Remarkably Stable β-Hairpin Peptide

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The methylation of lysine in histone proteins is a posttranslational modification that functions in the histone modulated process of chromatin condensation.[1] This phenomenon is associated with epigenetic silencing and, in a broader sense, has implications for designed mediators of protein−protein interactions. In particular, the methylated lysines in histone tails have been shown to trigger protein−protein interactions with several proteins containing the chromodomain. In each case, the binding of the $Lys(Me)_n^+$ (n = 1−3) occurs inside an aromatic pocket composed of three Trp and Tyr side chains.[1d] We have previously reported a β-hairpin peptide that is stabilized by a specific cation−π interaction between Trp2 and Lys9 in which the ϵ-methylene of Lys9 is packed against the face of the aromatic ring.[2] We report here the effects of lysine methylation on the magnitude of this cation−π interaction and the stability of the designed β-hairpin as a model system for Lys methylation in histone proteins. In the process, we have produced a remarkably stable β-hairpin that may be the most stable designed β-hairpin reported to date.

Peptides **1** and **2** (Figure 1) possess a Trp residue that is oriented diagonally in the hairpin with respect to a cationic residue. We have previously shown that when X = Lys, these two residues are in close proximity and interact in a favorable manner via a cation−π interaction, resulting in stabilization of the folded peptide.[2] The overall charge of peptides **1** and **2** is +3, lending increased water solubility and decreased aggregation to both. The peptides also include an Asn-Gly sequence, which has been shown to promote hairpin formation via a type I′ turn.[3] The peptides were synthesized by Fmoc solid-phase peptide synthesis, with the trimethylated lysine hairpin synthesized following the procedure of Kretsinger and Schneider.[4] Peptides were characterized by MALDI mass spectrometry and NMR as reported previously.[2] Numerous NOEs between cross-strand pairs of side chains were observed, consistent with β-hairpin formation.[5]

Both downfield shifting of the β-sheet H_α protons and the separation of the Gly H_α protons have been shown to correlate with the extent of folding in β-hairpins.[6] Inspection of the H_α chemical shifts relative to unfolded controls indicates that both peptides **1** and **2** take on a β-hairpin structure (Figure 2), as downfield shifting of the strand residues by $\geq$0.1 ppm is taken to represent a well-folded β-hairpin. Moreover, the H_α shifts indicate peptide **2** is more folded than peptide **1** at all positions along the strand, with the exception of the residues nearest the termini, which are typically frayed.[7]

The extent of folding of peptides **1** and **2** was quantified from the Gly H_α splitting relative to the fully folded and random coil control compounds, peptides **3**−**7**, as described previously.[2] Peptides **1** and **2** are estimated to be 78% and 92% folded, respectively.[8] This translates into ΔG_f values of −0.75 and −1.45 kcal/mol.[5] Hence, methylation increases the overall stability of the β-hairpin by about −0.7 kcal/mol. This is remarkable given the modest changes in the hairpin sequence. Analysis of the side-chain−side-chain interaction via double mutant cycles,[9] using Val and Ser as the control residues in positions 2 and 9,[2] gives a value of −0.4 ± 0.1 kcal/mol for the Lys···Trp interaction and −1.0 ± 0.1 kcal/mol for the $Lys(Me)_3^+$···Trp interaction. Thus, the change in stability of the β-hairpin is primarily due to the increase in the magnitude of the cation−π interaction upon methylation.

(a) Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-**X**-Ile-Leu-Gln-NH_2
1: X = Lys; **2**: X = $Lys(Me)_3^\oplus$

(b) Ac-Cys-Arg-Trp-Val-Glu-Val-Asn-Gly-Orn-**X**-Ile-Leu-Gln-Cys-NH_2
3: X = Lys; **4**: X = $Lys(Me)_3^\oplus$

(c) **5** Ac-Asn-Gly-Orn-Lys-Ile-Leu-Gln-NH_2
6 Ac-Asn-Gly-Orn-$Lys(Me)_3^\oplus$-Ile-Leu-Gln-NH_2
7 Ac-Arg-Trp-Val-Glu-Val-Asn-Gly-NH_2

Figure 1. (a) β-hairpin peptides **1** and **2**. (b) Control peptides for the fully folded state cyclized with a Cys−Cys disulfide bond between the underlined residues. (c) Control peptides for the unfolded state.

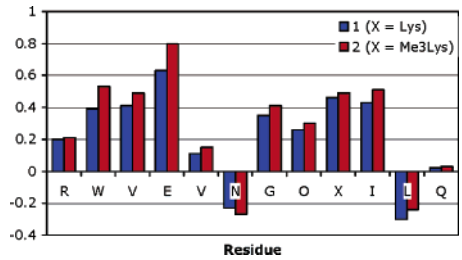

Figure 2. H_α shifts of peptides **1** and **2**. The Gly bars reflect the H_α separation in the hairpin. Conditions: 50 mM sodium acetate-d_4, pH 4.0 (uncorrected) at 298 K, referenced to DSS.

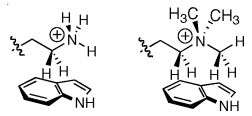

Figure 3. Possible interaction geometries for Trp···Lys and Trp···$Lys(Me_3)^+$ interactions.

We have previously demonstrated that the Lys residue of peptide **1** interacts preferentially with the face of the Trp ring through its polarized ϵ-methylene group (Figure 3).[2] Analysis of the $Lys(Me)_3^+$ side-chain upfield shift from peptide **2** indicates there is significant interaction of both the ϵ-methylene and its methylammonium group with the indole ring (Figure 4). In peptide **1**, the ϵ-methylene group is upfield shifted by 0.4 ppm, whereas in peptide **2**, it is shifted by almost 1 ppm. The methyl groups in $Lys(Me)_3^+$ are also significantly shifted by about 0.6 ppm, indicating they are also in close proximity to the face of the indole ring (Figure 3).

The Leu11 side chain, which is cross-strand from Trp, is also significantly upfield shifted at its γ and δ positions, indicating packing against the Trp ring.[5] However, the degree of upfield shifting of the Leu changes little between peptides **1** and **2**, indicating that, as a whole, the peptide experiences only minor conformational changes upon methylation of the Lys residue.

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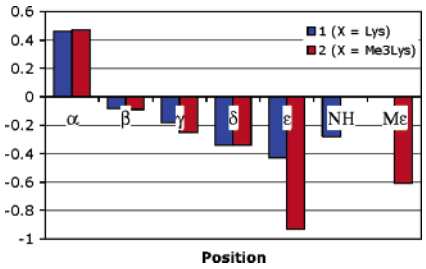

Figure 4. Side-chain chemical shifts of Lys (peptide **1**) and Lys(Me)$_3^+$ (peptide **2**). Conditions: 50 mM sodium acetate-d_4, pH 4.0 (uncorrected) at 298 K, referenced to DSS.

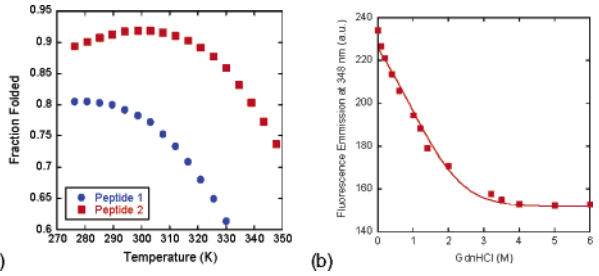

Figure 5. (a) Thermal denaturation of peptides **1** (circles) and **2** (squares) as determined by NMR. Conditions: 50 mM sodium acetate-d_4, pH 4.0 (uncorrected) at 298 K, referenced to DSS. (b) Chemical denaturation of peptide **2** as determined by change in Trp fluorescence with addition of guanidinium hydrochloride (GdnHCl). Conditons: 50 mM sodium phosphate buffer, pH 6.0 (uncorrected) at 298 K.

Table 1. Thermodynamic Parameters[a] for Folding at 298 K[10]

peptide	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/mol K)	$\Delta C_p°$ (cal/mol K)
1	−2.8	−6.8	−163
2	−0.2	4.3	−241

[a] Determined from the temperature dependence of the Gly chemical shift from 0 to 60 °C for peptide **1** and from 0 to 80 °C for peptide **2**.

Thermal denaturation of peptides **1** and **2** reveals that methylation of Lys results in a remarkably stable β-hairpin (Figure 5a). Other reported well-folded β-hairpins with high thermal stabilities such as trpzip4[10] and HP5W4,[11] both of which contain a four-Trp cluster, have similar stabilities at 298 K (92% and 96% folded, respectively) to that of peptide **2**. However, both denature more quickly at higher temperatures. For example, at 75 °C, peptide **2** is 74% folded, while trpzip4 is about 40% folded and HP5W4 is about 60% folded. This implies that peptide **2** has a higher melting temperature than either previously reported sequence. Notably, peptide **2** accomplishes high thermal stability without the Trp cluster that stabilizes both trpzip4 and HP5W4.

Fitting of the thermal denaturation of peptides **1** and **2** with the van't Hoff equation indicates that the methylation of Lys makes the folding of peptide **2** more entropically favorable and less enthalpically favorable than folding of the unmethylated peptide (Table 1).[6a] It appears that the methylation of Lys creates a tighter hydrophobic cluster with the Trp, resulting in a more favorable entropic term, which in turn enhances the observed strength of the cation−π interaction. The observed decrease in enthalpy with methylation can be attributed to a decrease in the electrostatic component of the interaction due to larger surface area over which the positive charge is distributed. The net increased magnitude of the cation−π interaction between tetraalkylammonium guests and cyclophane hosts versus dialkylammonium guests in aqueous solution has been documented by Dougherty and co-workers.[12] This effect has been attributed to the added hydrophobic component to the cation−π interaction upon increased methylation of the cation, which is in agreement with our thermodyamic analysis of peptides **1** and **2**.

We also performed a chemical denaturation of the β-hairpin with GdnHCl (Figure 5b). This denaturation plot clearly demonstrates the two-state nature of unfolding of the β-hairpin. Fitting results in a ΔG_f of −1.3 kcal/mol with $m = 0.8$.[13] This is in reasonable agreement with the ΔG_f determined from Gly chemical shifts, particularly given the error associated with the chemical denaturation resulting from the lack of an initial baseline.[13]

In conclusion, an investigation of the influence of Lys methylation on a cation−π interaction has resulted in the synthesis of an extremely stable β-hairpin peptide. We expect that further exploration of this sequence will not only help elucidate the role of Lys methylation in the function of histone tails but will also be of use to the engineering of β-turns in designed proteins and mediators of protein−protein interactions. Investigation of the effects of Lys monomethylation, dimethylation, and acylation upon similar β-hairpin systems is currently underway.

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Supporting Information Available: Synthesis, NMR assignments, NOEs, and thermodynamic measurements of reported compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(7) Note that H_α for Asn 6 is upfield shifted due to its position in the turn, and H_α for Leu 11 is upfield shifted due to ring current effects by the cross-strand Trp residue.
(8) The fraction folded as determined by the average H_α shift corresponds very well to that of the Gly splitting, with values of 77% and 90% for peptides **1** and **2**, respectively. See Supporting Information.
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