J|A|C|S
ARTICLES

Published on Web 06/10/2010

Dueling Post-Translational Modifications Trigger Folding and Unfolding of a β-Hairpin Peptide

Alexander J. Riemen and Marcey L. Waters*

Department of Chemistry, CB 3290, University of North Carolina, Chapel Hill, North Carolina 27599

Received February 5, 2010; E-mail: mlwaters@email.unc.edu

Abstract: Protein post-translational modifications (PTMs) are used in nature as a means of turning on or off a myriad of biological events. Methylation of lysine and phosphorylation of serine are important PTMs in the histone code found to modulate chromatin packing, which in turn affects gene expression. The design of peptides that fold into secondary structures can help to further our understanding of complex protein interactions. Here we report the design of the Trpswitch peptide sequence that folds into a moderately stable β-hairpin structure in aqueous solution and show that the stability of the structure can be tuned by incorporation of dimethyllysine or phosphoserine. Dimethylated Trpswitch results in an increase in β-hairpin stability, while phosphorylated Trpswitch is unstructured at neutral pH. When both modifications are incorporated into Trpswitch, a less stable β-hairpin structure is observed. This system provides a model to demonstrate how multiple PTMs may work in concert or against each other to influence structure.

Introduction

In biology, many cellular processes are regulated using chemical modification to proteins, resulting in activation or deactivation of a specific function. Gene regulation can be controlled by modification of the histone proteins that package DNA. Currently, there is extensive work on understanding how modifications to histone proteins affect gene transcription often referred to as the "histone code".[1,2] Not only do these modifications affect cell differentiation, it is becoming clear that altered histone modification profiles are present in diseases.[3] In fact, misregulation of histone modifications is involved in malaria[4] and mental disorders[5,6] and is being extensively studied in cancers.[3] Increased understanding of the histone code not only will further our understanding of fundamental biological processes but also may lead to new insights for treatment of diseases.

Two prevalent modifications to the N-terminal tail of the H3 histone protein known to affect gene transcription are phosphorylation of serine 10 and methylation of lysine 9.[7] Methylation of lysine 9 of the H3 histone triggers binding of heterochromatin protein 1 (HP1), which results in a restructuring of the chromatin structure that inhibits gene transcription. The methylated lysine binds through an aromatic cage in HP1 which only binds to di- and trimethylated lysine.[8] This binding is driven by cation$-\pi$ interactions between methylated lysine and the aromatic cage.[9] Work from the Allis laboratory has shown that binding of HP1 to histone 3 is inhibited when serine 10 of histone 3 is phosphorylated, thus allowing for transcription to occur.[7] The phosphorylation of serine 10 on histone 3 disrupts a favorable hydrogen bond with a glutamic acid residue in the bind groove of HP 1 and results in a charge$-$charge repulsion.[7] More recently, characterization of Chp1 chromodomain, another methylated lysine 9 recognition domain that utilizes an aromatic binding pocket, has shown that phosphorylation of serine 10 strongly reduces the Chp1 binding affinity to the histone 3 tail.[10]

While phosphorylation is a widely recognized PTM, until recently, Lys methylation was only known to occur in histone proteins. With the recent discovery of methylated Lys in non-histone proteins,[11] the question arises as to whether the opposing effects of neighboring Lys methylation and Ser (or Thr or Tyr) phosphorylation may be a general post-translational switching mechanism. There are now a number of model systems that have investigated the effect of a single PTM on peptide or protein folding,[12$-$26] but little work has focused on the collective

(1) Strahl, B. D.; Allis, C. D. *Nature* **2000**, *403*, 41–45.
(2) Jenuwein, T.; Allis, C. D. *Science* **2001**, *293*, 1074–1080.
(3) Lennartsson, A.; Ekwall, K. *Biochim. Biophys. Acta—Gen. Subj.* **2009**, *1790*, 863–868.
(4) Ralph, S. A.; Scherf, A. *Curr. Opin. Microbiol.* **2005**, *8*, 434–440.
(5) Waggoner, D. *Semin. Pediatr. Neurol.* **2007**, *14*, 7–14.
(6) Peedicayil, J. *Indian J. Med. Res.* **2007**, *126*, 105–11.
(7) Fischle, W.; Tseng, B. S.; Dormann, H. L.; Ueberheide, B. M.; Garcia, B. A.; Shabanowitz, J.; Hunt, D. F.; Funabiki, H.; Allis, C. D. *Nature* **2005**, *438*, 1116–22.
(8) Jacobs, S. A.; Khorasanizadeh, S. *Science* **2002**, *295*, 2080–2083.
(9) Hughes, R. M.; Wiggins, K. R.; Khorasanizadeh, S.; Waters, M. L. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 11184–11188.
(10) Schalch, T.; Job, G.; Noffsinger, V. J.; Shanker, S.; Kuscu, C.; Joshua-Tor, L.; Partridge, J. F. *Mol. Cell* **2009**, *34*, 36–46.
(11) Huang, J.; Berger, S. L. *Curr. Opin. Gen. Dev.* **2008**, *18*, 152–158.
(12) Shental-Bechor, D.; Levy, Y. *Curr. Opin. Struct. Biol.* **2009**, *19*, 524–533.
(13) Andrew, C. D.; Warwicker, J.; Jones, G. R.; Doig, A. J. *Biochemistry* **2002**, *41*, 1897–1905.
(14) Errington, N.; Doig, A. J. *Biochemistry* **2005**, *44*, 7553–8.
(15) Fujitani, N.; Kanagawa, M.; Aizawa, T.; Ohkubo, T.; Kaya, S.; Demura, M.; Kawano, K.; Nishimura, S.; Taniguchi, K.; Nitta, K. *Biochem. Biophys. Res. Commun.* **2003**, *300*, 223–229.
(16) Garcia-Alai, M. M.; Gallo, M.; Salame, M.; Wetzler, D. E.; McBride, A. A.; Paci, M.; Cicero, D. O.; de Prat-Gay, G. *Structure* **2006**, *14*, 309–319.
(17) Szilak, L.; Moitra, J.; Krylov, D.; Vinson, C. *Nat. Struct. Biol.* **1997**, *4*, 112–4.
(18) Szilak, L.; Moitra, J.; Vinson, C. *Protein Sci.* **1997**, *6*, 1273–83.

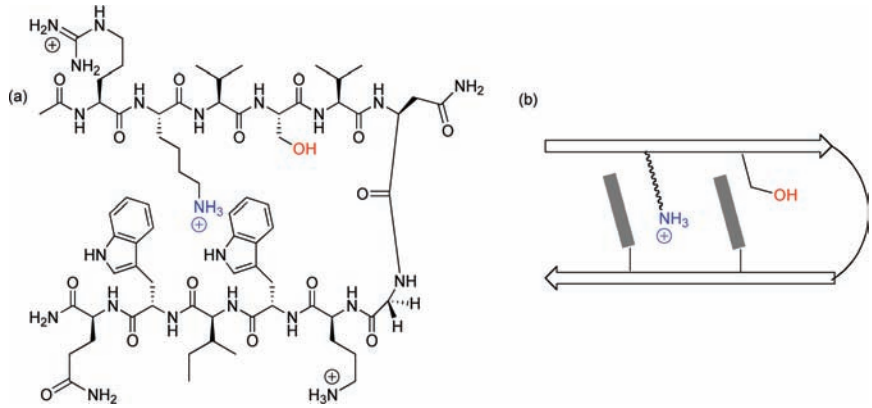

Figure 1. (a) Structure of the Trpswitch series of peptides. The ammonium group of lysine at position 2 in blue is dimethylated in Trpswitch-Me2 and Trpswitch-Me2/PO3 peptides. The hydroxyl group of serine at position 4 in red is phosphorylated in Trpswitch-PO3 and Trpswitch-Me2/PO3 peptides. (b) Schematic representation of the Trpswitch series of peptides demonstrating the interdigitating nature of residues 2, 4, 9, and 11. Gray bars represent Trp.

impact of multiple PTMs that appear to be critical in the histone code. Thus, using phosphorylation and methylation, we have designed a molecular switch peptide to investigate the combined effect of these modifications in a model system. These studies provide fundamental insight into how PTMs can affect protein structure and folding.

Results

System Design. The Trpswitch β-hairpin peptide was based on previously reported β-hairpin systems.[27] The structure of the Trpswitch peptides is given in Figure 1. The lysine at position 2 is located on the non-hydrogen-bonding (NHB) face of the β-hairpin cross-strand from a tryptophan pocket. Because the side chains of the β-hairpin interdigitate, Lys2 is wedged between the two Trp side chains, whereas Ser4 is packed against the opposite side of Trp9 (Figure 1b). Thus, direct interaction between Lys2 and Ser4 or phosphoserine (pSer) 4 is not possible in the folded state.

Placing Lys across from a pair of Trp residues was found to be highly stabilizing in the previously reported Trp pocket peptide;[27] however, now the tryptophan pocket is oriented on the C-terminal chain of the β-hairpin instead of the N-terminal chain. Dimethylated lysine is known to have a more favorable interaction with the tryptophan cleft in the Trp pocket peptides than Lys,[27] so it is expected that the incorporation of dimethyllysine (KMe2) at position 2 will increase overall folding. The Ser at position 4 on the NHB face is located cross-strand from Trp9 and is not expected to have any unfavorable interactions until it is phosphorylated, causing a disruption in the β-hairpin structure as seen in similar β-hairpin systems.[28]

Trpswitch Peptide Structure Studies. Structural studies were performed on the Trpswitch peptide and its variants using NMR spectroscopy and circular dichroism (CD). Downfield chemical shifts of the α-hydrogens (Hα) from random coil values are indicative of β-hairpin structure and can be used to determine the extent of folding.[29−32] A downfield shift of $\geq$0.1 ppm of at least three consecutive residues from the random coil value indicates β-sheet structure (Figure 2a).[30] The peptides Trpswitch, Trpswitch-Me2, and Trpswitch-Me2/PO3 all exhibit downfield shifting of at least 0.1 ppm for residues in the strands with the exception of Ser4 and Val5, which are upfield shifted due to shielding from cross-strand indole rings of Trp at position 9. The peptide Trpswitch-PO3 is shown to be unstructured with little difference from random coil values. The extent of folding into a β-hairpin structure was quantified using two methods described in the Experimental Section involving the splitting observed for the diastereotopic glycine α-protons in the turn and the extent of Hα shifting per residue compared to the fully folded cyclic control.[31−33] Using these methods, Trpswitch is calculated to be approximately 65% folded in aqueous solution (Table 1). Trpswitch-PO3 folding is negligible, and the peptide is considered unstructured. Incorporation of pSer results in a net destabilization of at least 2.0 kcal/mol relative to the unmodified Trpswitch. Trpswitch-Me2 is calculated to be approximately 86% folded, which is a 0.7 kcal/mol increase in stability (Table 1). The doubly modified peptide Trpswitch-Me2/PO3 is calculated to be about 30% folded and has approximately a 0.9 kcal/mol decrease in stability when compared to the unmodified Trpswitch (Table 1).[34]

CD was also employed to confirm the β-sheet structure of these peptides (Figure 2b). The β-sheet structure is characterized

(19) Kiehna, S. E.; Laughrey, Z. R.; Waters, M. L. *Chem. Commun.* **2007**, 4026–4028.
(20) Hughes, R. M.; Waters, M. L. *J. Am. Chem. Soc.* **2005**, *127*, 6518–9.
(21) Hughes, R. M.; Waters, M. L. *J. Am. Chem. Soc.* **2006**, *128*, 12735–12742.
(22) Hughes, R. M.; Waters, M. L. *J. Am. Chem. Soc.* **2006**, *128*, 13586–13591.
(23) Laughrey, Z. R.; Kiehna, S. E.; Riemen, A. J.; Waters, M. L. *J. Am. Chem. Soc.* **2008**, *130*, 14625–14633.
(24) Pons, J.; Evrard-Todeschi, N.; Bertho, G.; Gharbi-BenaroUs, J.; Benarous, R.; Girault, J. P. *Peptides* **2007**, *28*, 2253–2267.
(25) Megy, S.; Bertho, G.; Gharbi-Benarous, J.; Baleux, F.; Benarous, R.; Girault, J. P. *Peptides* **2005**, *26*, 227–241.
(26) Tokmakov, A. A.; Sato, K. I.; Fukami, Y. *Biochem. Biophys. Res. Commun.* **1997**, *236*, 243–7.
(27) Riemen, A. J.; Waters, M. L. *Biochemistry* **2009**, *48*, 1525–1531.
(28) Riemen, A. J.; Waters, M. L. *J. Am. Chem. Soc.* **2009**, *131*, 14081–14087.
(29) Syud, F. A.; Stanger, H. E.; Gellman, S. H. *J. Am. Chem. Soc.* **2001**, *123*, 8667–8677.
(30) Sharman, G. J.; Griffiths-Jones, S. R.; Jourdan, M.; Searle, M. S. *J. Am. Chem. Soc.* **2001**, *123*, 12318–12324.
(31) Griffiths-Jones, S. R.; Maynard, A. J.; Searle, M. S. *J. Mol. Biol.* **1999**, *292*, 1051–69.
(32) Syud, F. A.; Espinosa, J. F.; Gellman, S. H. *J. Am. Chem. Soc.* **1999**, *121*, 11577–11578.
(33) It should be noted that using these methods to calculate the fraction folded assumes a two-state system between the folded and unfolded states. The folding data from Hα shifts and Gly splitting, which provide information about the folding of the strands and the turn, respectively, are in good agreement, supporting a two-state system. Nonetheless, thermal denaturation studies indicate that folding and unfolding are not highly cooperative, since a sharp transition from folded to unfolded is not observed. .

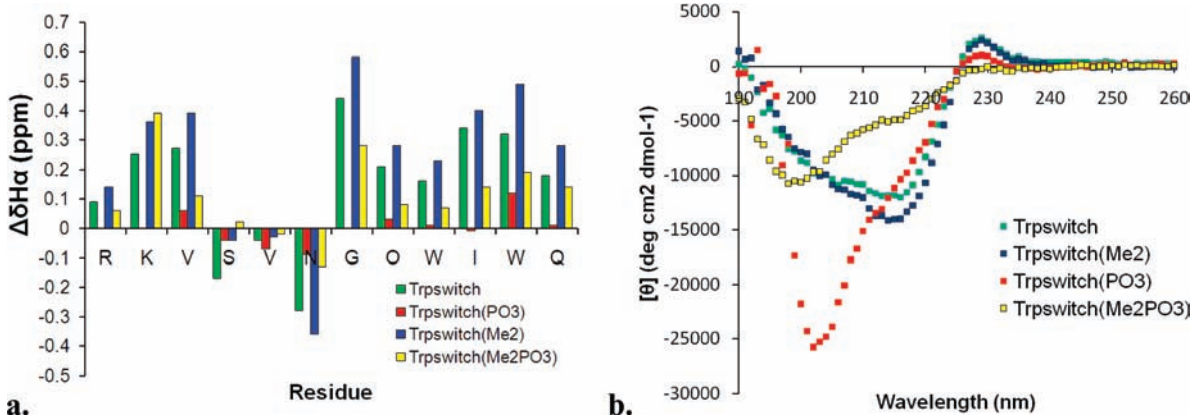

Figure 2. (a) Hα chemical shift differences from random coil controls. The Gly bars reflect the Hα splitting. Conditions: 293 K, 50 mM potassium phosphate in pD 7.0 buffer (uncorrected), referenced to DSS. (b) Circular dichroism spectral comparison of Trpswitch peptides at 298 K in 10 mM sodium phosphate pH 7.0 buffer.

Table 1. Fraction Folded and ΔG of Folding for β-Hairpin Peptides[a]

peptide	fraction folded (Gly splitting)[b]	fraction folded (Hα)[c]	ΔG(folding) (kcal/mol)	ΔG(Trpswitch-*x*−Trpswitch) (kcal/mol)
Trpswitch	0.68 ± 0.01	0.65 ± 0.02	−0.37 ± 0.02	
Trpswitch-PO3	0 ± 0.01	0.05 ± 0.07	1.7 ± 1.0	2.07 ± 1.0
Trpswitch-Me2	0.89 ± 0.01	0.86 ± 0.08	−1.11 ± 0.08	−0.73 ± 0.08
Trpswitch-Me2/PO3	0.43 ± 0.01	0.30 ± 0.08	0.5 ± 0.2	0.87 ± 0.2

[a] Values calculated from data obtained at 20 °C, 50 mM potassium phosphate-d_2, pD 7.0 (uncorrected), referenced to DSS. [b] Error determined by chemical shift accuracy on the NMR spectrometer. [c] Average of the Hα values from Val3, Val5, Orn8, and Ile10. The standard deviation is also given.

by a minimum between 210 and 215 nm, while the random coil structure is characterized by a minimum around 198 nm. The spectra of Trpswitch and Trpswitch-Me2 have large minima at 215 nm, confirming that these are well-folded β-hairpins. Trpswitch-Me2 is more folded than Trpswitch by CD, which correlates well with the NMR data. Trpswitch-PO3 has a large minimum at 198 nm, which is expected since this peptide is unfolded by NMR. The CD spectrum of Trpswitch-Me2/PO3 appears as a mixture between unfolded and β-sheet structure with a minimum around 198 nm and a shoulder at 215 nm that is consistent with the NMR data estimating that this peptide is only 30% folded.

Methylation of Lysine Results in Increased β-Hairpin Stability. The unmodified Trpswitch peptide is moderately folded, allowing for its stability to be increased or decreased upon modification. Incorporation of KMe2 results in a more stable β-hairpin as was seen in the similar Trp pocket peptide.[27] The increase in β-hairpin stability in the Trpswitch-Me2 peptide is due to more favorable interaction of the dimethylammonium group with the two cross-strand Trp indole rings. This is observed in NMR by the upfield shifting of the KMe2 side residues relative to random coil values (Figure 3). The greater upfield shifting observed indicates increased interaction of the lysine side chain with the tryptophan pocket. Comparison of the extent of side chain residue upfield shifting of lysine 2 between Trpswitch and Trpswitch-Me2 also shows an interesting trend: the δ- and ε-methylene protons in Trpswitch experience approximately the same amount of upfield shifting, and the γ-methylene experiences little upfield shifting, whereas Trpswitch-Me2 shows a progressive increase in upfield shifting of the

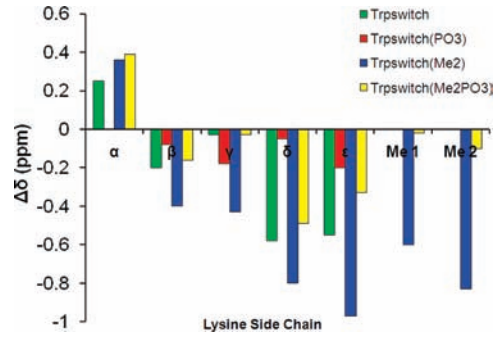

Figure 3. Lysine 2 side chain chemical shift from random coil values. Conditions: 20 °C, 50 mM potassium phosphate-d_2, pD 7.0 (uncorrected), referenced to DSS.

methylene hydrogens from the γ- to ε-position. This difference in upfield shifting along the lysine side chain suggests that lysine and dimethylated lysine interact differently with the cross-strand tryptophans. Trpswitch-Me2/PO3 also exhibits some upfield shifting of dimethylated lysine but to a much lesser extent compared to Trpswitch and Trpswitch-Me2, which suggests that this lysine interacts to some extent with the tryptophans, retaining some β-hairpin-like structure, which correlates with Hα shift and CD data. The lysine in Trpswitch-PO3 exhibits some slight upfield shifting that is difficult to interpret given the minimal folding of this peptide.

NOESY an ROESY NMR experiments were also performed on the more stable Trpswitch and Trpswitch-Me2 peptides to further confirm β-hairpin formation and specific side chain interactions (Figure 4).[35] Trpswitch has cross-strand NOEs between Trp9 and Lys2 as well as Ser4. Interaction between residues 2 and 9 is expected to be quite favorable due to the twist of the β-hairpin which has been observed in similar model β-hairpins.[20−22,27,36−38] The cross-strand NOEs between these residues further confirm proper strand register. It appears that Trp11 has little contact with Lys2, or else the nature of this interaction is highly dynamic, resulting in no observed long-range NOEs. Trpswitch-Me2 exhibits more cross-strand interac-

(34) For comparison, we found no significant difference in the Hα chemical shifts of the unfolded peptides corresponding to residues 1−7, indicating that the modifications did not measurably affect the unfolded state. See the Supporting Information.

(35) NOESY experiments gave a larger amount of cross-peaks than ROESY experiments, but the ROEs that were observed were consistent with the corresponding NOEs observed for each peptide. .

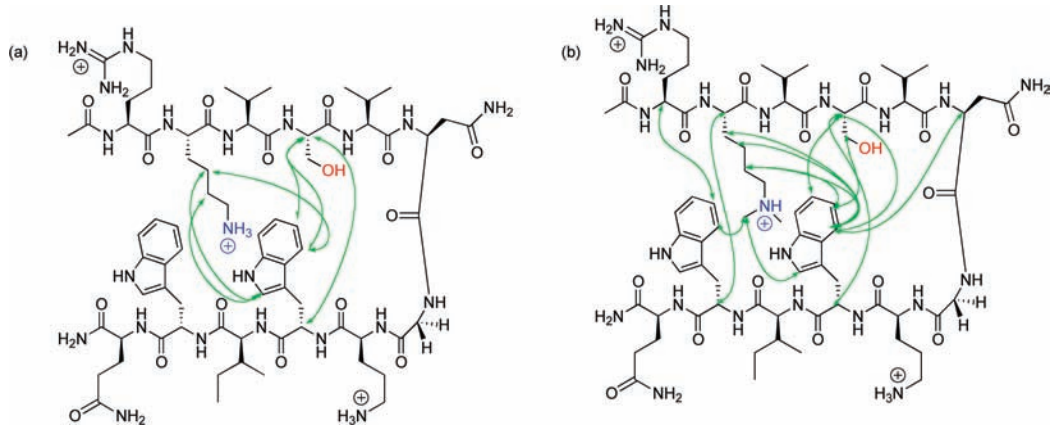

Figure 4. NOEs of cross-strand residues on the NHB face in (a) Trpswitch and (b) Trpswitch-Me2.

Table 2. Thermodynamic Parameters for Folding at 298 K for Trpswitch and Trpswitch-Me2 Peptides[a]

peptide	$\Delta H°$ (kcal/mol)	$\Delta S°$ (cal/(mol K))	$\Delta C_p°$ (cal/(mol K))	ref
Trp K pocket	-10.9 ± 0.8	-28 ± 3	-100 ± 21	27
Trp KMe pocket	-2.8 ± 0.2	-1.6 ± 0.7	-311 ± 8	27
Trpswitch	-6.88 ± 0.8	-22 ± 0.2	-55 ± 9	this work
Trpswitch-Me2	-8.1 ± 0.1	-23.9 ± 0.4	-60 ± 11	this work

[a] Conditions: 50 mM sodium acetate-d_4, pH 4.0 (uncorrected), referenced to DSS. Error obtained through thermal data fitting of eq 4 (see the Experimental Section).

tions between the lysine side chain and the two tryptophans, suggesting a more rigid and stable β-hairpin structure than that of the unmethylated Trpswitch. Some of the cross-strand NOEs observed for Trp9 and Lys2 are different between Trpswitch and Trpswitch-Me2, suggesting that the tryptophans adopt different conformations in the two peptides, in agreement with the lysine upfield shifting data.

Thermal denaturations were performed on the well-folded peptides Trpswitch and Trpswitch-Me2 to obtain thermodynamic parameters for folding and gain insight into the driving forces involved in β-hairpin stabilization. Peptide unfolding was monitored by NMR (Figure 4) using the extent of glycine splitting to determine the fraction folded as described in the Experimental Section. The data were fit to obtain the $\Delta H°$, $\Delta S°$, and $\Delta C_p°$ of folding using the method reported by Searle (eq 4 in the Experimental Section) (Table 2).[39] Trpswitch-Me2 has a larger enthalpic component than the unmodified Trpswitch and a similar entropic penalty for folding. The increased enthalpy of folding for Trpswitch-Me2 can be explained by a stronger cation$-\pi$ interaction between the tryptophan cleft and the dimethyllysine relative to unmethylated lysine. A cation$-\pi$ interaction of the positively charged lysine with tryptophan has been shown to be a major stabilizing force in many β-hairpins.[20,37,40,41] In these previously reported systems, methylation of lysine results in a change of the driving force for folding from enthalpic to entropic due to more favorable hydrophobic packing of the methyl groups with the tryptophan along with an increased number of favorable conformations. However, this is not what is observed in the Trpswitch system. The cation$-\pi$ interaction is stronger with dimethyllysine in Trpswitch-Me2, which is indicated by the more favorable $\Delta H°$ of folding as well as greater upfield shifting of the dimethylated lysine side chains. This discrepancy may be due to the positioning of the tryptophan cleft and the lysine, where in the Trp Pocket system the tryptophan cleft is located on the N-terminal strand of the hairpin and the lysine is close to the more ordered turn region.[27] The lysine and the tryptophans may not adopt an optimal cation$-\pi$ interaction in Trpswitch until it is methylated, thus promoting a more stable β-hairpin conformation. This hypothesis is supported by the NOE data for Trpswitch, which indicate that Lys2 has very few contacts with Trp9, suggesting that lysine is highly dynamic, and thus, a large entropic penalty is observed in the more stable Trpswitch-Me2, which must order this side chain to obtain a favorable interaction.

Destabilization of Trpswitch by Phosphoserine. Peptide systems have been reported that exhibit a loss in secondary structure or local conformation due to phosphorylation of key residues.[13,16,17,26,28,42] Disruption of α-helical peptides has been observed when residues within the helix are phosphorylated.[13,17,26] For example, peptide studies on the PEST sequence in the E2 protein from the papillomavirus have shown that serine phosphorylation of this region destabilizes the intrinsic α-helical and polyproline II structure.[16] The destabilization that occurs when Ser4 is phosphorylated in Trpswitch-PO3 and Trpswitch-Me2/PO3 is primarily the result of unfavorable cross-strand anion$-$Trp interaction as was reported in similar β-hairpin systems.[28] The repulsive interaction can be attributed to an unfavorable anion$-\pi$ or charge$-$hydrophobic interaction between the negatively charged phosphate and the electron-rich and hydrophobic indole ring of the cross-strand tryptophan. Phosphorylation may also result in a change in structure due to stabilization of the unfolded state via a Lys2$-$pSer4 interaction that is not possible in the folded state. However, previous studies of phosphorylation in a peptide that contained the cross-strand Trp but without Lys at the $i-2$ position indicate that the Lys residue is not necessary for phosphorylation to induce unfolding.[28]

As was observed in previously reported phosphorylated hairpins,[28] the β-hairpin structure is not as destabilized in Trpswitch-PO3 and Trpswitch-Me2/PO3 at lower pH, where

(36) Cochran, A. G.; Skelton, N. J.; Starovasnik, M. A. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 5578–5583.
(37) Tatko, C. D.; Waters, M. L. *Protein Sci.* **2003**, *12*, 2443–2452.
(38) Tatko, C. D.; Waters, M. L. *Protein Sci.* **2004**, *13*, 2515–2522.
(39) Maynard, A. J.; Sharman, G. J.; Searle, M. S. *J. Am. Chem. Soc.* **1998**, *120*, 1996–2007.
(40) Hughes, R. M.; Benshoff, M. L.; Waters, M. L. *Chem.—Eur. J.* **2007**, *13*, 5753–5764.
(41) Hughes, R. M.; Kiehna, S. E.; Waters, M. L. *Biopolymers* **2005**, *80*, 497–497.

(42) It should be noted that phosphorylation also can stabilize the structure as well, making this post-translational modification very context dependent. See refs 13−15, 43, and 44 for examples. .

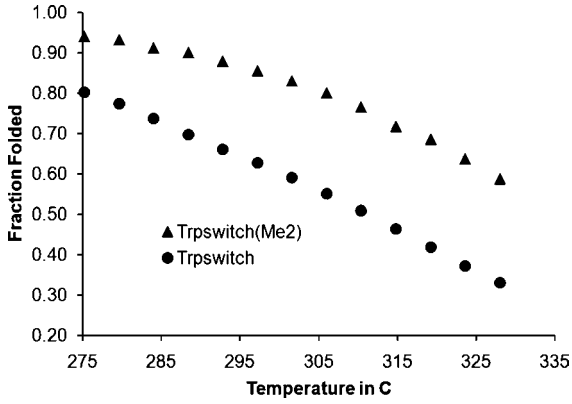

Figure 5. Thermal denaturation of Trpswitch (green) and Trpswitch-Me2 (blue). The values were calculated from data obtained in 50 mM sodium acetate-d_4, pH 4.0 (uncorrected), referenced to DSS. The fraction folded was calculated from the extent of Gly splitting.

the phosphate group is protonated, thus reducing its charge (Figure 6 and Table 3). Since the extent of folding is not completely regained at pH 1.2, where the phosphate group is neutral, phosphoserine could also prohibit the more stable hairpin structure through steric clash of the side chains or poorer β-sheet propensity.

Enzymatic Phosphorylation of Trpswitch. To assess whether the Trpswitch peptide can be enzymatically phosphorylated by a kinase, Trpswitch and Trpswitch-Me2 were incubated with the catalytic subunit of protein kinase A (PKA) from bovine heart. After 24 h all of the Trpswitch peptide was converted to Trpswitch-PO3, as confirmed by mass spectrometry. Trpswitch-Me2 was also phosphorylated, but the reaction was not complete after 24 h (see the Supporting Information). The reduced rate of phosphorylation could be due to the greater stability of the folded state or because KMe2 is a poor substrate for PKA. To address this, unstructured control peptides consisting of residues 1−7 of the Trpswitch and Trpswitch-Me2 sequences were also enzymatically phosphorylated by PKA (see the Supporting Information). Again, the unmodified lysine control peptide was completely phosphorylated after 24 h, while the dimethylated lysine control was slower to phosphorylate. This indicates that the secondary structure of the substrate does not affect the enzyme specificity of this system, but that KMe2 is not an optimal residue for substrate recognition of PKA, thus causing a decrease in the rate of phosphorylation. Thus, not only does methylation of Lys stabilize the folded state, but it has the ability to act as an inhibitor of phosphorylation in some cases, thereby maintaining the folded or "on" state. There are a number of such examples of cross-talk between different histone PTMs,[2,43−45] including the inhibition of histone H3 Ser10 phosphorylation by Ipl1/aurora kinase when H3 Lys9 is dimethylated.[46]

Discussion and Conclusions

Using a β-hairpin tryptophan cleft motif, we have designed a moderately folded β-hairpin peptide whose stability can be tuned on the basis of the type of post-translational modification present, recapitulating the effects of Lys methylation and Ser phosphorylation observed in the histone 3−HP1 chromodomain interaction.[7] The mechanism for stabilization of the folded state via enhanced cation−π interactions between the alkylated ammonium group and an aromatic pocket is similar to the mechanism employed in the native protein−protein interaction.[8,9] In contrast, the destabilization of the folded state via phosphorylation in this model system differs mechanistically from the native protein−protein interaction. In the native system, phosphorylation appears to inhibit binding of the HP1 chromodomain to the histone tail via electrostatic repulsion between the phospho-Ser and several glutamate residues in the chromodomain.[7] In the model system, however, unfolding is attributed to an unfavorable anion−Trp interaction.[28] This is an underexplored unfavorable interaction that may be important in other phosphorylation-induced signaling pathways. Nonetheless, this model system demonstrates molecular recognition principles by which two opposing PTMs can control biomolecular interactions.

In addition to providing insight into how two PTMs may act in concert to control biomolecular structure or function, this work provides a new mechanism with which to control folding that adds to the body of work on switchable peptides and proteins. Many conformational switch peptides have been designed as models for complex structural transitions found in nature, including peptides that switch between different folding motifs,[47−52] from a folded to unfolded state or vice versa,[53−55] or from a folded to an aggregated state.[53,56−65] Switchable systems have been developed to respond to a wide range of stimuli,[48,66] including pH,[47−50,53,56,60] temperature,[48,51,61] salt

(43) Cheung, P.; Tanner, K. G.; Cheung, W. L.; Sassone-Corsi, P.; Denu, J. M.; Allis, C. D. *Mol. Cell* **2000**, *5*, 905–915.
(44) Lo, W. S.; Trievel, R. C.; Rojas, J. R.; Duggan, L.; Hsu, J. Y.; Allis, C. D.; Marmorstein, R.; Berger, S. L. *Mol. Cell* **2000**, *5*, 917–926.
(45) Clayton, A. L.; Rose, S.; Barratt, M. J.; Mahadevan, L. C. *EMBO J.* **2000**, *19*, 3714–3726.
(46) Rea, S.; Eisenhaber, F.; O'Carroll, N.; Strahl, B. D.; Sun, Z. W.; Schmid, M.; Opravil, S.; Mechtler, K.; Ponting, C. P.; Allis, C. D.; Jenuwein, T. *Nature* **2000**, *406*, 593–599.
(47) Pagel, K.; Wagner, S. C.; Samedov, K.; von Berlepsch, H.; Bottcher, C.; Koksch, B. *J. Am. Chem. Soc.* **2006**, *128*, 2196–2197.
(48) Pagel, K.; Koksch, B. *Curr. Opin. Chem. Biol.* **2008**, *12*, 730–9.
(49) Mutter, M.; Gassmann, R.; Buttkus, U.; Altmann, K. H. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1514–1516.
(50) Mimna, R.; Camus, M. S.; Schmid, A.; Tuchscherer, G.; Lashuel, H. A.; Mutter, M. *Angew. Chem., Int. Ed.* **2007**, *46*, 2681–4.
(51) Ciani, B.; Hutchinson, E. G.; Sessions, R. B.; Woolfson, D. N. *J. Biol. Chem.* **2002**, *277*, 10150–10155.
(52) Ambroggio, X. I.; Kuhlman, B. *J. Am. Chem. Soc.* **2006**, *128*, 1154–1161.
(53) Mutter, M.; Chandravarkar, A.; Boyat, C.; Lopez, J.; Dos Santos, S.; Mandal, B.; Mimna, R.; Murat, K.; Patiny, L.; Saucede, L.; Tuchscherer, G. *Angew. Chem., Int. Ed.* **2004**, *43*, 4172–4178.
(54) Mizuno, T.; Suzuki, K.; Imai, T.; Kitade, Y.; Furutani, Y.; Kudou, M.; Oda, M.; Kandori, H.; Tsumoto, K.; Tanaka, T. *Org. Biomol. Chem.* **2009**, *7*, 3102–3111.
(55) Balakrishnan, S.; Zondlo, N. J. *J. Am. Chem. Soc.* **2006**, *128*, 5590–5591.
(56) Camus, M. S.; Dos Santos, S.; Chandravarkar, A.; Mandal, B.; Schmid, A. W.; Tuchscherer, G.; Mutter, M.; Lashuel, H. A. *ChemBioChem* **2008**, *9*, 2104–2112.
(57) Taniguchi, A.; Skwarczynski, M.; Sohma, Y.; Okada, T.; Ikeda, K.; Prakash, H.; Mukai, H.; Hayashi, Y.; Kimura, T.; Hirota, S.; Matsuzaki, K.; Kiso, Y. *ChemBioChem* **2008**, *9*, 3055–3065.
(58) Pagel, K.; Wagner, S. C.; Araghi, R. R.; von Berlepsch, H.; Bottcher, C.; Koksch, B. *Chem.—Eur. J.* **2008**, *14*, 11442–11451.
(59) Pagel, K.; Seri, T.; von Berlepsch, H.; Griebel, J.; Kirmse, R.; Bottcher, C.; Koksch, B. *ChemBioChem* **2008**, *9*, 531–536.
(60) Rajagopal, K.; Lamm, M. S.; Haines-Butterick, L. A.; Pochan, D. J.; Schneider, J. P. *Biomacromolecules* **2009**, *10*, 2619–2625.
(61) Pochan, D. J.; Schneider, J. P.; Kretsinger, J.; Ozbas, B.; Rajagopal, K.; Haines, L. *J. Am. Chem. Soc.* **2003**, *125*, 11802–11803.
(62) Haines, L. A.; Rajagopal, K.; Ozbas, B.; Salick, D. A.; Pochan, D. J.; Schneider, J. P. *J. Am. Chem. Soc.* **2005**, *127*, 17025–17029.
(63) Ozbas, B.; Kretsinger, J.; Rajagopal, K.; Schneider, J. P.; Pochan, D. J. *Macromolecules* **2004**, *37*, 7331–7337.
(64) Dublin, S. N.; Conticello, V. P. *J. Am. Chem. Soc.* **2008**, *130*, 49+.
(65) Kuhnle, H.; Borner, H. G. *Angew. Chem., Int. Ed.* **2009**, *48*, 6431–4.
(66) Chockalingam, K.; Blenner, M.; Banta, S. *Protein Eng. Des. Sel.* **2007**, *20*, 155–161.

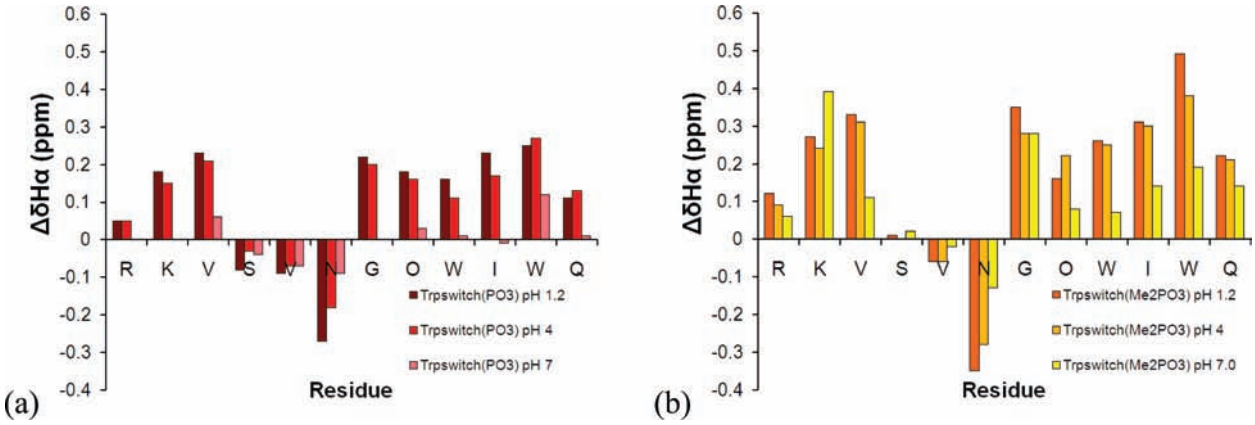

Figure 6. Hα chemical shift differences from random coil controls in varying pD for (a) Trpswitch-PO3 and (b) Trpswitch-Me2/PO3. The Gly bars reflect the Hα separation in the hairpin. At pD 7.0 the phosphate group has a −2 charge, at pD 4.0 a −1 charge, and at pD 1.2 a neutral charge. All NMR data were obtained at 20 °C.

Table 3. Fraction Folded of Phosphorylated Trpswitch Analogues[a]

	fraction folded		
peptide	pH 1.2	pH 4.0	pH 7.0
Trpswitch-PO3	0.51 ± 0.07	0.44 ± 0.10	0.05 ± 0.07
Trpswitch-Me2/PO3	0.60 ± 0.12	0.60 ± 0.13	0.30 ± 0.08

[a] Fraction folded determined from Val3, Gly 7, Orn 8, and Ile 10 Hα chemical shift data as described in the Experimental Section. Error calculated from the standard deviation of average residue fraction folded calculations.

concentration,[48,63] metal ions,[48,51,52,59,64,67,68] redox conditions,[69,70] binding,[54] and phosphorylation.[28,55,65] The switch peptide presented here demonstrates the ability to use opposing PTMs to control the extent of folding. The Trpswitch peptide lends itself as a starting point to design more complex peptide systems that rely on post-translational modifications to tune structure, resulting in a loss or gain of structure and/or function.

Experimental Section

Synthesis and Purification of Peptides. Peptides were synthesized by automated solid-phase peptide synthesis on an Applied Biosystems Pioneer peptide synthesizer using Fmoc-protected amino acids on a PEG-PAL-PS resin. Fmoc-[N]-protected and benzyl-[O]-protected phosphoserine and dimethylated Fmoc-protected lysine were purchased from AnaSpec. Activation of amino acids was performed with HBTU and HOBT in the presence of DIPEA in DMF. Peptide deprotection was carried out in 2% DBU (1,8-diazabicyclo[5.4.0]undec-7-ene) and 2% piperidine in DMF for approximately 10 min. Extended cycles (75 min) were used for each amino acid coupling step. All control peptides where acetylated at the N-terminus with 5% acetic anhydride and 6% lutidine in DMF for 30 min. Cleavage of the peptide from the resin was performed in 95:2.5:2.5 trifluoroacetic acid (TFA)/ethanedithiol or triisopropylsilane (TIPS)/water for 3 h. Ethanedithiol was used as a scavenger in for sulfur-containing peptides. TFA was evaporated, and cleavage products were precipitated with cold ether. The peptide was extracted into water and lyophilized. It was then purified by reversed-phase HPLC, using a Vydac C-18 semipreparative column and a gradient of 0−100% B over 40 min, where solvent A was 95:5 water/acetonitrile with 0.1% TFA and solvent B was 95:5 actonitrile/water with 0.1% TFA. After purification the peptide was lyophilized to powder and identified with ESI-TOF mass spectroscopy.

Cyclization of Peptides. Cyclic control peptides were cyclized by oxidizing the cysteine residues at the ends of the peptide via stirring in a 10 mM phosphate buffer (pH 7.5) in 1% DMSO solution for 9−12 h. The solution was lyophilized to a powder and purified with HPLC using the method described above.

CD Spectroscopy. CD spectroscopy was performed on an Aviv 62DS circular dichroism spectrophotometer. Spectra were collected from 260 to 185 nm at 25 °C with 1 s scanning.

NMR Spectroscopy. NMR samples were made to a concentration of 1 mM in D_2O buffered to pD 4.0 (uncorrected) with 50 mM NaOAc-d_3, 24 mM AcOH-d_4 and 0.5 mM DSS or pD 7.0 (uncorrected) with 50 mM $KPOD_4$ and 0.5 mM DSS. Samples were analyzed on a Varian Inova 600 MHz instrument. One-dimensional spectra were collected by using 32K data points and between 8 and 128 scans using 1.5 s presaturation. Two-dimensional total correlation spectroscopy (TOCSY) and nuclear Overhauser spectroscopy (NOESY) experiments were carried out using the pulse sequences from the ChemPack software. Scans in the TOCSY experiments were taken from 16 to 32 in the first dimension and from 64 to 128 in the second dimension. Scans in the NOESY experiments were taken from 32 to 64 in the first dimension and from 128 to 512 in the second dimension with mixing times of 200−500 ms. All spectra were analyzed using standard window functions (sine bell and Gaussian with shifting). Presaturation was used to suppress the water resonance. Assignments were made by using standard methods as described by Wüthrich.[71] All experiments were run at 293 K except for thermal melt experiments.

Determination of the Fraction Folded. To determine the unfolded chemical shifts, 7-mers were synthesized as unstructured controls and cyclic peptides were synthesized for fully folded states. The chemical shifts for residues in the strand and one turn residue were obtained from each 7-mer peptide. The chemical shifts of the fully folded state were taken from the cyclic peptides. The fraction folded on a per residue basis was determined from the following equation:

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

where δ_{obsd} is the observed Hα chemical shift, δ_{100} is the Hα chemical shift of the cyclic peptides, and δ_0 is the Hα chemical shift of the unfolded 7-mers. The overall fraction folded for the entire peptide was obtained by averaging the fraction folded of resides Val3, Lys8, and Ile10. These residues are in hydrogen-

(67) Wright, C. M.; Heins, R. A.; Ostermeier, M. *Curr. Opin. Chem. Biol.* **2007**, *11*, 342–346.
(68) Cerasoli, E.; Sharpe, B. K.; Woolfson, D. N. *J. Am. Chem. Soc.* **2005**, *127*, 15008–15009.
(69) Schenck, H. L.; Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 12487–12494.
(70) Pandya, M. J.; Cerasoli, E.; Joseph, A.; Stoneman, R. G.; Waite, E.; Woolfson, D. N. *J. Am. Chem. Soc.* **2004**, *126*, 17016–17024.
(71) Wüthrich, K. *NMR of Proteins and Nucleic Acids*; Wiley: New York, 1986.

bonded positions and have been shown to be the most reliable in determining the fraction folded.[32] The overall fraction folded was also determined using the extent of Hα glycine splitting observed in the turn residue Gly7 given in the following equation:

$$\text{fraction folded} = \Delta\delta_{\text{Gly,obsd}}/\Delta\delta_{\text{Gly,100}} \quad (2)$$

where $\Delta\delta_{\text{Gly,obsd}}$ is the difference in the glycine Hα chemical shifts observed and $\Delta\delta_{\text{Gly,100}}$ is the difference in the glycine Hα chemical shifts of the cyclic peptides.

The ΔG of folding at 298 K for the Trpswitch peptides was calculated using the following equation, where f is the fraction folded:

$$\Delta G = -RT\ln(f/(1-f)) \quad (3)$$

Determination of Thermodynamic Parameters. Variable-temperature NMR was used to determine the thermodynamic parameters of the peptide folding. A temperature range of 275−351 K was explored in 5 K increments using a Varian Inova 600 MHz spectrometer. Temperature calibration was performed with ethylene glycol and methanol standards by using standard macros in Varian software. The change in the glycine chemical shift difference was followed with temperature. The fraction folded of the peptide was plotted against temperature, and the curve was fitted by using the following equation:[39]

$$\text{fraction folded} = (\exp[x/RT])/(1+\exp[x/RT]) \quad (4)$$

where

$$x = (T[\Delta S^{\circ}_{298} + \Delta C_p^{\circ}] - [\Delta H^{\circ}_{298} + \Delta C_p^{\circ}\{T-298\}])$$

Enzymatic Phosphorylation of Trpswitch. The peptides Trpswitch and Trpswitch-Me2 were enzymatically phosphorylated with protein kinase A catalytic subunit from bovine heart purchased from Sigma-Aldrich. Lyophilized protein kinase A was reconstituted in PKA buffer (10 mM magnesium chloride, 6 mg/mL dithiothreitol, 50 mM potassium phosphate buffer at pH 6.9) and allowed to sit for 10 min before addition of substrate. A 100 μM concentration of peptide substrate was reacted with 1 mM adenosine triphosphate and 100 UN PKA in PKA buffer at 30 °C for 24 h. One UN is defined as transfer of 1.0 pmol of phosphate from [γ-^{32}P]ATP to hydrolyzed, partially dephosphorylated casein per minute at pH 6.5 at 30 °C according to Sigma-Aldrich. Phosphorylation of peptides was determined by LC−MS.

Acknowledgment. We gratefully acknowledge support from the National Science Foundation (CHE-0716126).

Supporting Information Available: Table listing the NMR chemical shift assignments for all peptides and figures showing the LC−MS trace of enzymatic phosphorylation of Trpswitch hairpins and HPLC traces of PKA phosphorylation on control peptides **1** and Me2-**1**. This material is available free of charge via the Internet at http://pubs.acs.org.

JA101079Z