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Conformational Changes Associated with Post-Translational Modifications of Pro^{143} in Skp1 of *Dictyostelium*—A Dipeptide Model System

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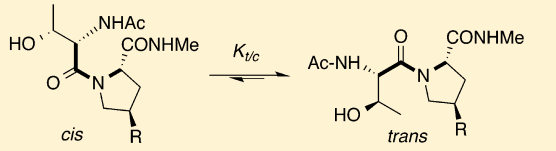
Supporting Information

ABSTRACT: Prolyl hydroxylation and subsequent glycosylation of the E3^{SCF} ubiquitin ligase subunit Skp1 affects its conformation and its interaction with F-box proteins and, ultimately, O_2-sensing in the organism. Taking a reductionist approach to understand the molecular basis for these effects, a series of end-capped Thr-Pro dipeptides was synthesized, tracking the sequential post-translational modifications that occur in the protein. The conformation of the pyrrolidine ring in each compound was gauged via coupling constants ($^3J_{H\alpha,H\beta}$) and the electronegativity of the Cγ-substituents by chemical shifts (^{13}C). The equilibrium between the *cis*–*trans* conformations about the central prolyl peptide bond was investigated by integration of signals corresponding to the two species in the ^{1}H NMR spectra over a range of temperatures. These studies revealed an increasing preference for the *trans*-conformation in the order Pro < Hyp < [α-(1,4)GlcNAc]Hyp. Rates for the forward and reverse reactions, determined by magnetization transfer experiments, demonstrated a reduced rate for the *trans*-to-*cis* conversion and a significant increase in the *cis*-to-*trans* conversion upon hydroxylation of the proline residue in the dipeptide. NOE experiments suggest that the Thr side chain pushes the sugar away from the pyrrolidine ring. These effects, which depended on the presence of the *N*-terminal Thr residue, offer a mechanism to explain altered properties of the corresponding full-length proteins.



at 298K, K_{tc} = 2.3 (R = H); 8.7 (R = OH) and 13.2 [R = α-(1,4)-GlcNAcO]

INTRODUCTION

The process whereby proteins are polyubiquitinated and delivered to the 26S proteasome for degradation involves several steps and is fundamental to the maintenance of healthy levels of all proteins in eukaryotic organisms. A prominent really interesting new gene (RING)-type third order (E3) ubiquitin ligase, referred to as the Cullin-RING-Ligase-1 (CRL1) or the Skp1-Cullin-F-box (SCF) complex, consists of several subunits including the S-phase kinase-associated protein 1 (Skp1).[1] In *Dictyostelium discoideum*, the proline residue at position 143 of Skp1 is hydroxylated by a cytoplasmic prolyl hydroxylase (PhyA).[2] We have recently confirmed that the hydroxylation, by analogy to the hypoxia inducible factor α (HIFα) prolyl hydroxylases, delivers (2*S*,4*R*)-4-hydroxyproline (Hyp).[3] Hydroxylation of Skp1 is important for the proper oxygen-dependence of differentiation of the organism into a fruiting body to disperse spores.[4,5] Polypeptide *N*-acetylglucosaminyl-transferase 1 (Gnt1) transfers *N*-acetylglucosamine (GlcNAc) from uridine diphosphate (UDP) to Hyp^{143} of Skp1.[6,7] The installation of the first GlcNAc residue renders Skp1 subject to further glycosylation by the PgtA (β3GalT/α2FucT) and AgtA (αGalT) glycosyltransferases,[8−10] which modulate oxygen-dependence conferred by prolyl hydroxylation. The hypothesized terminal structure, illustrated in Figure 1, has been validated for all but the linkage of the terminal

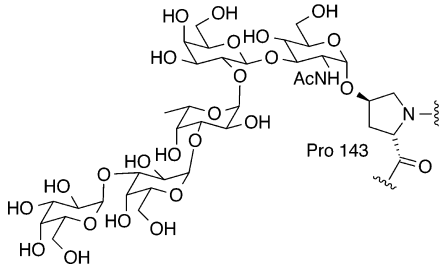


Figure 1. Glycosylated region of Skp1.

αGal.[10] Biophysical studies, viz. circular dichroism (CD) and small-angle X-ray scattering (SAXS), indicate that the αGlcNAc residue promotes α-helical secondary structure and increased order of the polypeptide.[11] These post-translational modifications occur in other unicellular eukaryotes, including *Toxoplasma gondii*.[8,12,13]

While a fundamental impact of glycosylation on protein structure and function has been widely appreciated for many years, the empirical basis for structural changes is less well understood. Previous studies have focused on the more

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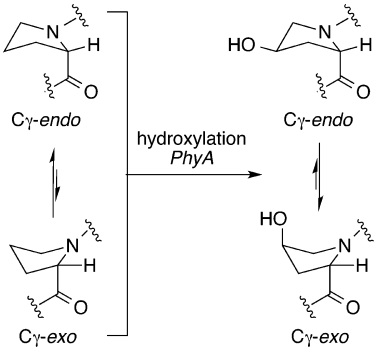
ubiquitous *O*-glycosidic linkages to serine and threonine residues.[14−18]

Prior to hydroxylation, the pyrrolidine ring of the Pro[143] residue is likely to adopt a Cγ-*endo* conformation (Scheme 1).

Scheme 1. Pyrrolidine Conformation



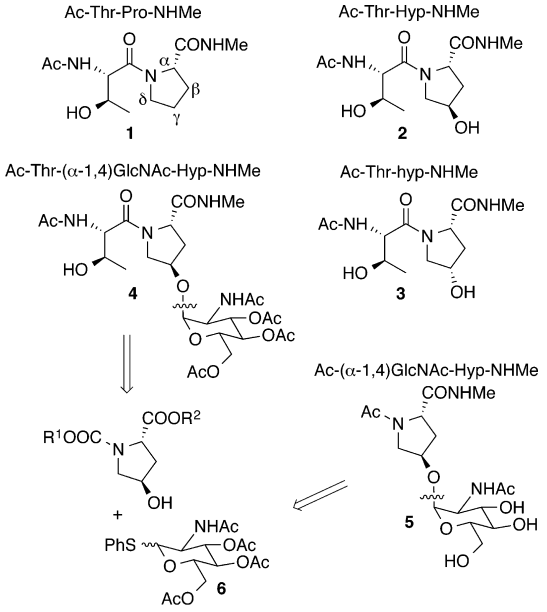
X-ray crystallographic studies show that the Thr-Pro[143] peptide bond favors the *trans* conformation (ω = 180°) when the Skp1 proteins from *Saccharomyces cerevisiae* or *Arabidopsis thaliana* are complexed to an F-box protein.[19,20] This *cis*−*trans* isomerism might be important in Skp1 folding, perhaps mediated by a Thr-Pro prolyl isomerase.[21] By analogy to recent findings in the orthologous human HIFα,[22,23] upon hydroxylation by a *trans*-4-prolylhydroxylase, there could be dramatic changes in the topology of this region of Skp1, viz. pyrrolidine ring-flip to the Cγ-*exo* conformation and a reinforced preference for a *trans* amide bond (ω = 180°), which may in turn influence processing by Gnt1 and F-box protein binding. With a GlcNAc residue installed, torsion angles about the glycosidic bond will contribute to recognition by other proteins, including F-box proteins and the β3GalT/α2FucT that introduces the next two monosaccharides.[12,24,25] These interrelated conformational effects may provide the trigger for α-helix formation in Skp1.

Hydroxyproline glycosides are common in plants; however, no examples have been reported to-date in mammalian systems.[26] In 2007, Schweizer and co-workers reported on the thermodynamics and kinetics of *cis*−*trans* isomerization of a family of proline derivatives, Ac-Pro*-NHMe where Pro* was L-proline (Pro), *trans*-4-hydroxy-L-proline (Hyp), *trans*-4-*tert*-butoxy-L-proline, α-(1,4)-galactosyl-*trans*-4-hydroxy-L-proline [α-(1,4)-Gal-Hyp], and β-(1,4)-galactosyl-*trans*-4-hydroxy-L-proline [β-(1,4-Gal)-Hyp].[27] The surprising result of this work was that, within experimental error, the equilibrium constants and rate constants were similar for all compounds. We report herein that for a dipeptide that emulates the Thr[142]-Pro[143] sequence in Skp1 there are noteworthy changes that correlate with recent findings on the F-box binding capacity of the isoforms of Skp1.

RESULTS AND DISCUSSION

We prepared dipeptides **1**−**4** (Scheme 2) that track the initial post-translational modifications of Skp1 and incorporate the preceding Thr residue. We end-capped the dipeptides to mimic the amide backbone of the protein and to eliminate electrostatic effects that arise from ionizable groups.[28,29] At the outset, we did not know the configuration at Cγ of the hydroxyproline residue and thus included *cis*-4-hydroxy-L-proline (hyp) in our repertoire of proline analogues. Specifically

Scheme 2. Dipeptides Synthesized



compounds **2** and **3** were synthesized and used to confirm the 4*R* configuration of the hydroxproline residue in Skp1.[3] In addition, Ac-[α-(1,4)GlcNAc]Hyp-NHMe (**5**) was prepared. This compound represents the GlcNAc analogue of Ac-[α-(1,4)Gal]-Hyp-NHMe described previously by Schweizer and co-workers.[27] Full details of the synthesis of the compounds depicted in Scheme 2 are provided in the Supporting Information. In the case of glycosides **4** and **5**, glycosylation was performed with thioglycoside donor **6** using NIS/AgOTf activation[30,31] and an appropriate carbamate protected hydroxyproline ester.

Analysis of the ^{1}H NMR ^3J coupling constants $J_{\alpha\beta1}$ and $J_{\alpha\beta2}$ (α, β etc. as defined for compound **1** in Scheme 2) gave an indication of the conformational preference of the pyrrolidine ring in each compound (Table 1). According to the literature,

Table 1. *J*-Values and Chemical Shifts Derived from ^{1}D NMR Spectra of Compounds 1−5

compound	$J_{\alpha\beta1}$, $J_{\alpha\beta2}$ (Hz)	δ Cγ (ppm)
Ac-Thr-Pro-NHMe (**1**)	nd[a]	23.6[b]
Ac-Thr-Hyp-NHMe (**2**)	9.7, 7.8	69.7[c]
Ac-Thr-hyp-NHMe (**3**)	9.2, 4.6	69.5 (67.5)[d]
Ac-Thr-[α-(1,4)GlcNAc]Hyp-NHMe (**4**)	9.7, 8.1	75.7[c]
Ac-[α-(1,4)GlcNAc]-Hyp-NHMe (**5**)	8.5, 8.5	74.8 (74.0)[d]

[a]Not determined due to overlap between Pro Hα and Thr Hβ. [b]While two species are evident, the two Cα signals appear to be coincident. [c]On the time scale of the ^{13}C NMR experiment a single, averaged species was observed. [d]The value in parentheses represents a signal due to the minor conformation.

in a Cγ-*exo* ring pucker both *J*-values are in the specific limiting range of 7−11 Hz, sometimes even converging to produce an apparent triplet.[32,33] In the Cγ-*endo* ring pucker there are two quite different dihedral angles between Hα and the neighboring Hβ protons, giving rise to distinct coupling constants in the ranges 2−3 and 6−10 Hz. In the case of a twist conformation, dynamically averaged values of ∼7 Hz are anticipated. Thus, the pyrrolidine rings in compounds **2**, **4**, and **5** adopt a Cγ-*exo* conformation. The $J_{\alpha\beta}$ values could not be determined for

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compound **1** due to overlap between Pro Hα and Thr Hβ signals. For dipeptide **3** containing hyp, the *J*-values do not fit either profile but are in agreement with those reported for Ac-hyp-OMe by Owens et al.[34] and may signify a slightly different conformation, viz. Cβ-*exo*, Cδ-*exo*, or related twist confromations.[35]

The electron-withdrawing nature and *R*-configuration of substituents at Cγ are attributed with favoring the Cγ-*exo* conformation of the pyrrolidine ring and *inter alia* the *trans* conformation of the preceding prolyl peptide bond. The ^{13}C NMR chemical shift for Cγ has been used to assess the strength of the electron-withdrawing substituents; our results appear in Table 1. Hydroxylation at Cγ leads to a significant downfield shift from 23.6 to 69.7 ppm. Glycosylation further deshields the Cγ nucleus from the magnetic field, leading to an additional 6 ppm shift downfield upon glycosylation of hydroxyproline, in accord with the observations of Schweizer and co-workers upon α-D-galactosylation.[27] The *J*-values and Cγ chemical shifts reported in Table 1 are very similar to those reported for the Ac-Pro*-NHMe series,[27] implying that the additional Thr residue does not affect pyrrolidine ring conformation.

The thermodynamics of the *cis* → *trans* isomerization were studied by NMR as described previously.[36,37] $K_{t/c}$ values were determined at 5° temperature intervals from 25 to 85 °C, by integration of well-resolved signals for protons associated with the *cis* and *trans* conformations. The derived van't Hoff plots are depicted in Figure 2. All peptides demonstrated a decrease in $K_{t/c}$ with an increase in temperature, and the linear nature of the plots indicates that $\Delta H°$ and $\Delta S°$ are independent of temperature.

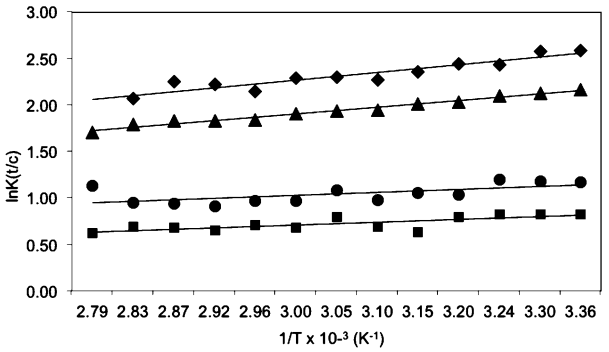


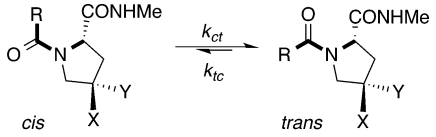
Figure 2. Van't Hoff plots for compounds **1** (■), **2** (▲), **4** (◆), and **5** (●).

In the case of the dipeptides, one of the most useful signals in these studies, vis-à-vis resolution and intensity, was the doublet at ~δ1.25 ppm, attributable to the methyl group (Hγ) of the Thr residue. At lower temperatures, there was excellent resolution between the upfield (*cis*) and downfield (*trans*) signals. An increase in temperature was accompanied by a move toward coalescence of the two signals; however, complete coalescence was not observed within the temperature range accessible in D_2O.[38] Broadening and partial coalescence of the signals in the case of glycosylated dipeptide **4** led to omission of the 85 °C data point (Figure 2), since integration of the two signals was not possible.

The incidence of *cis* prolyl peptide bonds, when preceded by a Thr residue, has been estimated at 2.9% based on crystallographic data for proteins.[39] In shorter peptides, without secondary structure, the percentage is higher.[40] Larger acyl groups *N*-terminal to the Pro residue have a larger steric bias toward the *trans* conformation. This notwithstanding, $K_{t/c}$ for dipeptide **1** is low in the context of other dipeptides we have studied: Ac-Gly-Pro-NHMe ($K_{t/c}$ 5.5 in D_2O at 298 K) and Ac-Phe-Pro-NHMe ($K_{t/c}$ 2.2).[36] The latter is expected to favor the *cis* conformation due to an aromatic−proline interaction.

As anticipated,[37,38,41] hydroxylation of the proline residue led to a significant shift in favor of the *trans* peptide bond (Scheme 3, Table 2). This arises from stereoelectronic effects that favor a

Scheme 3. *cis* → *trans* Isomerization of Prolyl Amide Bond



1 R = Ac-Thr; X = Y = H
2 R = Ac-Thr; X = OH; Y = H
4 R = Ac-Thr; X = [(α-1,4)GlcNAc]O; Y = H
5 R = CH_3; X = [(α-1,4)GlcNAc]O; Y = H
7 R = CH_3; X = Y = H
8 R = CH_3; X = OH; Y = H
9 R = CH_3; X = [(α-1,4)Gal]O; Y = H

Table 2. Thermodynamics Parameters for Compounds 1, 2, 4, 5, and 7−9

compound	$K_{t/c}$ (298 K)	$\Delta H°$ (kcal mol^{-1})	$\Delta S°$ (cal mol^{-1} K^{-1})	$\Delta G°$(298 K) (kcal mol^{-1})
1	2.3	−0.64	−0.52	−0.49
2	8.7	−1.52	−0.80	−1.28
4	13.2	−1.73	−0.72	−1.52
5	3.3	−0.68	+0.06	−0.70
7[a]	3.2	−0.95	−0.87	−0.65
8[a]	3.9	−1.33	−1.76	−0.81
9[a]	3.7	−1.27	−1.64	−0.78

[a]Data from ref 27.

Cγ-*exo* conformation of the pyrrolidine ring that, in-turn, aligns the peptide backbone for a stabilizing n → π^* interaction[42,43] in the *trans* conformation. Formation of the α-glycoside further accentuates this effect. This second result contrasts with earlier reports from Schweizer's group that showed both α- and β-galactosylation of Ac-Hyp-NHMe[27] or Ac-Hyp-OMe[34] to have essentially no impact on the thermodynamics or kinetics of the *cis* → *trans* equilibrium.

To investigate the mechanism behind the differences in equilibrium constants for compounds **1**−**5**, kinetics of the prolyl peptide bond isomerization were studied by magnetization transfer NMR experiments.[44,45] Experiments were performed at elevated temperatures (60−80 °C) because at lower temperatures the rates are too slow to be determined by this method. Related experiments have been performed in unbuffered D_2O,[46−48] and Reimer et al. demonstrated that for end-capped pentapeptide Ac-Ala-Thr-Pro-Ala-Lys-NH_2, the rate of *cis*−*trans* isomerization was independent of pH across the 1.8−10.8 range and a variety of temperatures.[39] This was shown to be a general phenomenon across a series of pentapeptides without ionizable side chains. The CIFIT program[49] was used to estimate the rate constants for the *trans* → *cis* process. The reverse reaction rates (*cis* → *trans*) were derived therefrom in conjunction with the equilibrium constants. Data were fitted according to the Eyring equation[50] to afford the activation energy data presented in Table 3.

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Table 3. Activation Parameters for *trans* → *cis* and *cis* → *trans* Isomerization about the Prolyl Amide Bond[a]

	trans → cis				cis → trans			
compound	k_{tc} (s^{-1})	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (cal mol^{-1} K^{-1})	$\Delta G^{\ddagger}$ (kcal mol^{-1})	k_{ct} (s^{-1})[c]	$\Delta H^{\ddagger}$ (kcal mol^{-1})	$\Delta S^{\ddagger}$ (cal mol^{-1} K^{-1})	$\Delta G^{\ddagger}$ (kcal mol^{-1})
1[b]	*0.38*[e]	*9.86*	*−32.6*	*19.57*	*0.72*[f]	*9.86*	*−30.6*	*18.98*
2[b]	*0.22*[e]	*18.12*	*−8.3*	*20.59*	*1.33*[f]	*16.96*	*−8.0*	*19.34*
5[b]	*0.34*[e]	*18.9*	*−5.8*	*20.63*	*0.94*[f]	*17.90*	*−6.8*	*19.93*
7[b,d]	0.31	21.3	1.4	20.9	0.81	20.6	1.2	20.2
8[b,d]	0.25	21.1	0.5	21.0	0.73	20.2	0.1	20.2
9[c,d]	0.27	20.6	1.0	21.3	0.83	22.4	6.2	20.6

[a]The italic values are data determined in the current work; data below those in italics come from the literature[27] for comparative purposes. [b]Unbuffered D_2O. [c]Phosphate buffer, pH 7.4. [d]Reference 27. [e]Measured by magnetization transfer. [f]Calculated from $K_{t/c}$ and k_{tc}.

Upon hydroxylation there is a decrease in the rate of conversion of *trans* → *cis* ∼20% in the case of Ac-Pro-NHMe (**8** vs **7**)[27] and ∼40% at the dipeptide level (**2** vs **1**). The *cis* → *trans* rates do not follow the same trend; there is a ∼10% decrease in rate from **7** to **8**,[27] but a ∼80% increase from **1** to **2**. This accounts for the more pronounced increase in $K_{t/c}$ in the dipeptide series. Indeed, Larive and Rabenstein also concluded that large $K_{t/c}$ values for prolyl peptide bond isomerization in oxytocin and arginine−vasopressin were also due to an increase in the rate of the forward reaction, rather than an inherent increase in the stability of the *trans* conformation, relative to smaller molecules.[46] With the increase in electronegativity of the 4*R* substituent, there is presumably an increase in the sp^3-character and degree of pyramidalization of the pyrrolidine nitrogen that facilitates rotation about the C−N amide bond.[47,51,52] As originally demonstrated by Fischer et al.[51], a hydrogen bonding interaction between the pyramidalized nitrogen and the N**H** of the residue *C*-terminal to Pro (mimicked in our case by the N**H**Me) helps to lower the energy of the transition state. Model compounds in which this amide is substituted for by an ionizable carboxylate or an ester cannot emulate this feature of the peptide backbone.

In the proline methyl amides **7**−**9**, the free energy barriers to rotation about the amide bond are dominated by enthalpic factors. While entropy values are positive and close to zero for these amino acid derivatives, significant negative entropies of activation are observed for compounds **1**, **2**, and **5**. This is generally true of larger peptides[46] and reflects a higher degree of organization in the transition state for an extended peptide molecule.[28] Compound **1** shows very different activation enthalpy and entropy contributions, relative to compounds **2** and **4**. This must be attributable to the influence of the electron-withdrawing substituent on the energy and degree of organization of the transition state.

In the case of glycodipeptide **4** there was no *cis*/*trans* pair of resonances in the ^{1}H NMR spectrum with adequate resolution for the signal of one conformation to be selectively inverted without also inverting the signal of the other conformation. Shi et al. have previously acknowledged that not all peptides are amenable to rate determination in this manner.[47] Lubell and co-workers have used inversion of the C*α* *trans* signal in ^{13}C NMR to measure rates of *trans* → *cis* isomerization.[38,53] In the present case, a single set of resonances was observed for compound **4**, even at 70 °C. These experimental facts precluded magnetization transfer experiments that would have given insight into the impact of glycosylation on *cis*−*trans* isomerization kinetics at the dipeptide level.

The importance of *N*-acetyl groups of sugars in hydrogen bonding and peptide conformation has been commented on by Wong and co-workers[16] and also by Live et al.[54,55] The differences in $K_{t/c}$, k_{tc}, and k_{ct} between compound **5**, bearing a GlcNAc residue, and the Gal derivative **9** reported by Schweizer and co-workers[27] are almost within experimental error. The only notable difference is in the entropies of activation: the GlcNAc residue in **5** seems to incur a higher degree of order in the transition state for peptide bond isomerization than the Gal residue in **9**.

On the basis of GOESY experiments conducted for compound **9**, Owens et al. described a conformation in which the hydrophobic face of the galactose residue sits over the top of the pyrrolidine ring[27] (Figure 3). There were few crosspeaks

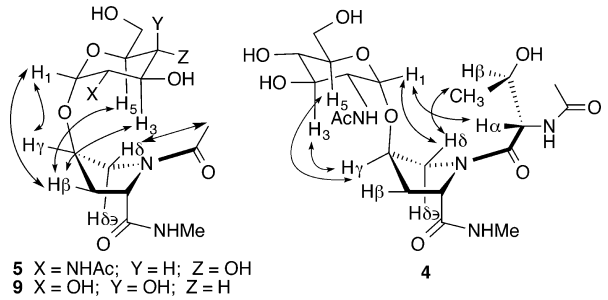

Figure 3. Conformation of compounds **4**, **5**, and **9**. Double-headed arrows indicate NOE correlations.

in the NOESY spectrum of compound **5**, in which Gal is substituted by GlcNAc. Weak crosspeaks potentially support a conformation similar to that of **9** but are ambiguous due to severe peak overlap in the 3.5−4.0 ppm region of the ^{1}H NMR spectrum. A more defined conformation for dipeptide **4** was discernible, based on NOESY crosspeaks summarized in Figure 3. One-dimensional GOESY experiments supported these conclusions (see Supporting Information). As depicted in Figure 3, it appears that the strong preference for the C*γ*-*exo* pyrrolidine ring and *trans* peptide bond (with alignment for the n → *π** backbone interaction) renders the threonine side chain partially over the pyrrolidine ring, pushing the sugar residue away.

To better understand the role of the threonine residue, we considered key chemical shift and coupling constant data for this residue. In compounds **1**, **2**, and **4**, the Thr C*α* and C*β* chemical shifts did not show much variation: 57.1−57.2 and 67.1−68.8 ppm, respectively. These are somewhat upfield of statistical average values,[56−58] as expected when the (*i* + 1) residue is Pro.[59] Amide proton (vide infra) and H*α* chemical shifts likewise showed little change upon C*γ* substitution: 8.15−8.26 and 4.58−4.61 ppm, respectively. These values are closer to those observed for random coil peptides than for *α*-helix or *β*-sheet. motifs.[55−57] There was an increase in $^3J_{NH,H\alpha}$ across the

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series at 295 K: 4.8 Hz (**1**), 6.9 Hz (**2**), and 7.8 Hz (**4**) reflecting a change in the theta (ϕ) dihedral angle.

^{1}H NMR spectra were acquired in 90% H_2O, 10% D_2O at neutral pH. Amide protons were assigned on the basis of TOCSY spectra. The C-terminal methyl amide (N**H**CH$_3$) gave rise to a broad signal with a similar chemical shift in all compounds. The GlcN**H**Ac amide in compounds **4** and **5** showed a typical ^3J coupling contant of 9−10 Hz to H2.[60] The AcN**H**Thr amide (in compounds **1**, **2**, and **4**) displayed a chemical shift of δ 8.1−8.2 ppm. A significant difference in chemical shift was observed for the Hδ that is *trans* to Hγ in compounds **4** (δ4.17 ppm) and **5** (δ3.79 ppm). This reflects the relatively deshielded environment in compound **4** where the polar Thr side chain is in close proximity, compared to compound **5** where Hδ is shielded by the hydrophobic face of the sugar. In all cases, variable temperature experiments showed typical temperature dependence for non-hydrogen bonded amides ($\Delta\delta < -4$ ppb K^{-1}). Thus, the strong conformational preferences appear to be due to steric and stereoelectronic effects and thereby provide a true baseline for moving forward to study larger peptides.

CONCLUSION

Reorganization of the pivotal Pro^{143} region of Skp1 is a recurring theme during interactions with F-box proteins. Recent CD and SAXS studies show that the secondary structure content of free Skp1 of *Dictyostelium* is sensitive to its glycosylation status which correlates with binding to a model F-box protein.[11] Crystal structures have demonstrated a well-folded state for Skp1 complexed with F-box proteins in other organisms.[19,20] The equivalent of Pro^{143} is highly conserved throughout phylogeny except for chordate animals. Even in organisms with multiple Skp1 genes, at least one copy of the Pro-containing Skp1 is retained. The amino acid at this position initiates the C-terminal α-helix. Even in Skp1 proteins where the equivalent of Pro^{143} is substituted by another amino acid, the theme of reorganization persists. A study by Tan et al. of OCP2 (an orthologue of Skp1 from the organ of Corti) concluded that binding to OCP1 (bearing an F-box motif) is accompanied by a substantial degree of refolding.[61] The *C*-terminal region is alternatively folded in a loop in one complex.[62]

Thus, a picture is emerging that Skp1 exists as an ensemble of conformations whose suitability to dock with F-box protein partners is under environmental regulation via effects of post-translational modifications to Skp1. Indeed, Zondlo and co-workers have recently uncovered the role of threonine/serine phosphorylation and GlcNAc attachment in mediating neighboring secondary structure.[63,64] The model we propose herein is analogous to the mechanism by which the Skp1 homologue elongin c selects its binding partners in a related E3 polyubiquitin ligase[65] and may allow for regulation of the rate or selectivity of polyubiquitination of target proteins with impact on development. Milner-White et al. observed that Pro residues at the N-terminus of α-helices always adopt *trans* prolyl peptide bonds and demonstrate a strong preference for the Cγ-*exo* pyrrolidine conformation.[66] The post-translational modifications to Pro^{143} enhance preferences for these conformational attributes and thereby offer an attractive mechanism to trigger global changes in Skp1 conformation and deserve further mechanistic studies.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures for preparation of compounds **1**−**6** and associated 1D ^{1}NMR spectra. Integration data for peptides **1**, **2**, **4**, and **5** are tabulated along with calculated thermodynamics parameters. For compounds **1**, **2**, and **5**, plots for magnetization transfer experiments are provided along with calculated kinetics parameters. TOCSY spectra and amide chemical shift changes as a function of temperature are provided for compounds **1**, **2**, **4**, and **5**. NOESY and pertinent GOESY spectra are provided for compounds **4** and **5**. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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