J|A|C|S
COMMUNICATIONS

Published on Web 02/02/2006

Electronic Control of Amide cis−trans Isomerism via the Aromatic−Prolyl Interaction

Krista M. Thomas, Devan Naduthambi, and Neal J. Zondlo*

Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716

Received December 1, 2005; E-mail: zondlo@udel.edu

The cis−trans isomerization of amide bonds results in large structural and functional changes in proteins and is a rate-limiting step in protein folding.[1,2] Strategies to control the cis−trans isomerization state are critical to answer fundamental questions in protein folding, protein function, and macromolecular complex formation.[3] Herein we demonstrate a novel electronic strategy to control cis−trans isomerization, based on the demonstration that interactions between aromatic residues and proline are tunable by aromatic electronics.

Cis amide bonds in proteins occur with the highest frequencies in aromatic−proline residue pairs, via the aromatic−prolyl interaction (Figure 1). Tyr−Pro and Trp−Pro sequences exhibit higher populations of cis amide bonds than Phe−Pro, suggesting that the aromatic−prolyl interaction depends on aromatic ring electronics and thus is a potentially tunable interaction.[4−6] In proteins, cis aromatic−prolyl amide bonds are stabilized by stacking of the aromatic ring and the proline ring, with the proline H_α, H_β, or H_δ centered under the aromatic ring.[7] In addition to the hydrophobic effect, the aromatic−prolyl interaction has been proposed to be stabilized by a C−H−π interaction, in which the aromatic ring donates electron density to the relatively electron-deficient C−H bonds.[6−9] This description suggests that electron-rich aromatic residues should stabilize this interaction and promote cis amide bonds. In contrast, electron-deficient aromatic residues should yield a less favorable aromatic−prolyl interaction and relatively favor trans amide bonds.

To address whether aromatic electronics may be used to control amide cis−trans isomerism, a series of peptides TXPN was synthesized, where X = electron-rich, electron-neutral, or electron-deficient aromatic residues (Figure 1).[10] As a control, the peptide containing the hydrophobically similar nonaromatic residue, cyclohexylalanine (Cha), was also synthesized.

All peptides containing aromatic residues favored the cis conformation relative to the control peptides TChaPN or TAPN (Table 1). Peptides containing electron-rich aromatic residues relatively favored cis amide bonds, with Trp > Tyr > Phe among canonical amino acids. In contrast, electron-deficient aromatic residues relatively disfavored the cis conformation, with 4-NO_2-phenylalanine and protonated 4-pyridylalanine the most trans-favoring residues. The population of cis isomer correlated with aromatic ring electronic density: a Hammett correlation was observed between $K_{trans/cis}$ and σ_{para} for 4-substituted phenylalanines (Figure 2).[11] These data are consistent with a C−H−π interaction stabilizing the aromatic−prolyl interaction and indicate that electronics may be used to tune peptide and protein structure and stability.[12,13]

To further tune cis−trans isomerism via the aromatic−prolyl interaction, we examined replacement of proline with conformationally biased 4-substituted proline derivatives (Figure 1).[14] In peptides with electron-rich aromatic residues, replacement of proline with 4*S*-fluoroproline (flp) further stabilized the cis conformation

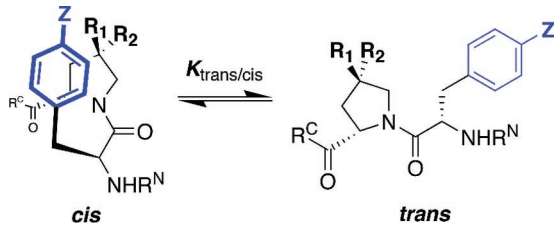

Figure 1. The cis−trans isomerization of an aromatic−prolyl amide bond, with the cis-stabilizing aromatic−prolyl interaction represented. $K_{trans/cis}$ was examined in peptides TXPN, X = aromatic residue (4-substituted phenylalanines (4-Z-Phe), tryptophan, pentafluorophenylalanine, and pyridylalanine). Sites of electronic (Z) and stereoelectronic (R^1, R^2) tuning are indicated. Nomenclature of proline derivatives: Hyp (R^1 = OH, R^2 = H); Flp (4*R*-fluoroproline; R^1 = F, R^2 = H); flp (4*S*-fluoroproline; R^1 = H, R^2 = F). R^N = Ac−Thr−; R^C = −Asn−NH_2.

Table 1. Electronic Tuning of the Aromatic−Prolyl Interaction[a]

TXPN, X =	$K_{trans/cis}$	$\Delta G_{trans/cis}$ kcal mol^{-1}
4-H^+pyridyl−Ala	7.6	−1.19
4-pyridyl−Ala	5.7	−1.02
4-NO_2−Phe	5.5	−1.00
4-CF_3−Phe	4.7	−0.91
4-$^+NH_3$−Phe	4.4	−0.87
F_5−Phe	4.4	−0.87
4-I−Phe	4.1	−0.83
Phe (F)	3.2	−0.68
Tyr (Y)	2.7	−0.58
4-NH_2−Phe	2.1	−0.44
Trp (W)	1.8	−0.35
4-O^-−Phe	1.7	−0.31
Cha	8.0	−1.22
Ala (A)	10.7	−1.39

[a] NMR-derived data for TXPN peptides. $\Delta G = -RT \ln K_{trans/cis}$. Experiments were conducted at 23 °C in H_2O containing 5 mM phosphate and 25 mM NaCl.

(Table 2). In contrast, in peptides with electron-poor aromatic residues, replacement of proline with 4*R*-hydroxyproline (Hyp) or 4*R*-fluoroproline (Flp) increased $K_{trans/cis}$. Overall, by combining electronic and stereoelectronic approaches, cis−trans isomerism of aromatic−proline residue pairs was modulated by 2.0 kcal mol^{-1}. These results indicate that similar approaches may find broad utility in the control of protein stability and the development of novel inhibitors of protein−protein complexes.

The peptide TWflpN exhibited 60% cis amide bond and $^3J_{\alpha N}$ for Trp_{cis} = 4.2 Hz, corresponding to $\phi = -61°$,[15] a remarkable degree of ordering in a simple tetrapeptide. These data are consistent with the cis conformation adopting a type VI β-turn, an important conformation in protein−protein interfaces.[6,10,16,17]

In addition to restricted ϕ, several additional lines of evidence indicate that the cis conformation of TWflpN adopts a type VIa1 β-turn, which requires a cis amide bond between the i+1 and i+2 residues and defined backbone torsion angles at the i+1 ($\phi = -60°$,

10.1021/ja057901y CCC: $33.50

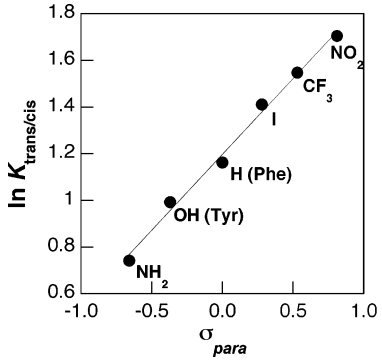

Figure 2. Hammett plot correlating aromatic amino acid side chain electronics (σ_{para} of Z for 4-Z-phenylalanines) with the amide cis−trans equilibrium of T(4-Z−Phe)PN peptides ($\rho = 0.29$, $R = 0.996$).

Table 2. Electronic and Stereoelectronic Tuning of Amide cis−trans Isomerism: NMR-Derived Data at 23 °C for TXProxN Peptides

peptide	$K_{trans/cis}$	$\Delta G_{trans/cis}$ kcal mol^{-1}
T(4-H$^+$pyridyl−Ala)FlpN	20.1	−1.76
T(4-NO_2−Phe)HypN	9.5	−1.32
TFPN	3.2	−0.68
TYflpN	1.5	−0.24
T(4-NH_2−Phe)flpN	1.0	0.00
TWflpN	0.65	+0.25

$\psi = +120°$) and $i+2$ ($\phi = -90°$, $\psi = 0°$) residues. The flp_{cis} H_α (3.50 ppm), H_β (0.66, 0.59 ppm), and H_δ (2.06, 2.06 ppm) chemical shifts are shifted substantially upfield compared to those in flp_{trans} (H_α 4.68; H_β 2.57, 2.47; H_δ 3.98, 3.92 ppm), as has been observed in type VI β-turns, consistent with a strong aromatic−prolyl interaction.[5,16] In addition, a series of ROEs consistent with a type VIa1 β-turn were observed, including a diagnostic ROE between Trp H_α and Asn H_N.[18] Moreover, the Trp and flp $^1J_H\alpha_C\alpha$ coupling constants, which can be correlated with ψ,[19] were consistent with near-ideal ϕ and ψ in TWflpN.[18] These data demonstrate that TWflpN adopts an electronically stabilized type VIa1 β-turn, without introducing steric bulk on proline.[20]

The thermodynamic basis of stabilization of the cis conformation by the aromatic−prolyl interaction was examined via temperature-dependent NMR.[5,14,18] At 298 K, enthalpy favors the cis conformation in both TWPN ($\Delta H = +0.28$ kcal mol^{-1}) and TWflpN ($\Delta H = +2.0$ kcal mol^{-1}), in contrast to the usual enthalpic stabilization of the trans conformation of an X-Pro bond (Ac−GP−OMe: $\Delta H = -1.27$ kcal mol^{-1}).[21]

Interactions between aromatic rings and proline rings are important stabilizing factors in protein structures and protein−protein complexes.[7,22] The data herein indicate that the interactions between aromatic residues and proline are driven significantly by π-electron donor−prolyl electron acceptor interactions that provide specific stabilization in addition to the classical hydrophobic effect.[2,5,8,10,12]

We have demonstrated broad control of cis−trans isomerism via electronic and stereoelectronic tuning of the aromatic−prolyl interaction. Given the importance of the interactions between aromatic and proline rings in biomolecular recognition and of cis−trans isomerism in protein folding, the results and approaches herein should find general application to control, probe, and understand protein structure and function.

Acknowledgment. We thank the University of Delaware and the NIH (T32 GM 08550) for support of this work.

Supporting Information Available: Experimental procedures, characterization data, CD data, and NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Reimer, U.; Fischer, G. *Biophys. Chem.* **2002**, *96*, 203−212. (b) Dugave, C.; Demange, L. *Chem. Rev.* **2003**, *103*, 2475−2532. (c) Andreotti, A. H. *Biochemistry* **2003**, *42*, 9515−9524. (d) Lorenzen, S.; Peters, B.; Goede, A.; Preissner, R.; Frommel, C. *Proteins: Struct., Funct., Bioinf.* **2005**, *58*, 589−595.

(2) Meyer, E. A.; Castellano, R. K.; Diederich, F. *Angew. Chem., Int. Ed.* **2003**, *42*, 1210−1250.

(3) Lummis, S. C. R.; Beene, D. L.; Lee, L. W.; Lester, H. A.; Broadhurst, R. W.; Dougherty, D. A. *Nature* **2005**, *438*, 248−252.

(4) (a) Grathwohl, C.; Wüthrich, K. *Biopolymers* **1976**, *15*, 2025−2041. (b) Yao, J.; Feher, V. A.; Espejo, B. F.; Reymond, M. T.; Wright, P. E.; Dyson, H. J. *J. Mol. Biol.* **1994**, *243*, 736−753. (c) Reimer, U.; Scherer, G.; Drewello, M.; Kruber, S.; Schutkowski, M.; Fischer, G. *J. Mol. Biol.* **1998**, *279*, 449−460. Electronic control of cis−trans isomerization in aryl amides: (d) Forbes, C. C.; Beatty, A. M.; Smith, B. D. *Org. Lett.* **2001**, *3*, 3595−3598. (e) Yamasaki, Y.; Tanatani, A.; Azumaya, I.; Saito, S.; Yamaguchi, K.; Kagechika, H. *Org. Lett.* **2003**, *5*, 1265−1267.

(5) Wu, W.-J.; Raleigh, D. P. *Biopolymers* **1998**, *45*, 381−394.

(6) Halab, L.; Lubell, W. D. *J. Am. Chem. Soc.* **2002**, *124*, 2474−2484.

(7) (a) Pal, D.; Chakrabarti, P. *J. Mol. Biol.* **1999**, *294*, 271−288. (b) Bhattacharyya, R.; Chakrabarti, P. *J. Mol. Biol.* **2003**, *331*, 925−940.

(8) (a) Toth, G.; Murphy, R. F.; Lovas, S. *Protein Eng.* **2001**, *14*, 543−547. (b) Brandl, M.; Weiss, M. S.; Jabs, A.; Sühnel, J.; Hilgenfeld, R. *J. Mol. Biol.* **2001**, *307*, 357−377. (c) Nakagawa, Y.; Irie, K.; Yanagita, R. C.; Ohigashi, H.; Tsuda, K.-I. *J. Am. Chem. Soc.* **2005**, *127*, 5746−5747.

(9) (a) Nishio, M.; Umezawa, Y.; Hirota, M.; Takeuchi, Y. *Tetrahedron* **1995**, *51*, 8665−8701. (b) Nishio, M. *Cryst. Eng. Commun.* **2004**, *6*, 130−158.

(10) This context has been identified to promote cis amide bonds: Meng, H. Y.; Thomas, K. M.; Lee, A. E.; Zondlo, N. J. *Biopolymers (Peptide Sci.)* **2006**, in press, DOI: 10.1002/bip.20382.

(11) For a recent example of a Hammett correlation of a π-facial interaction, see: Cockroft, S. L.; Hunter, C. A.; Lawson, K. R.; Perkins, J.; Urch, C. J. *J. Am. Chem. Soc.* **2005**, *127*, 8594−8595.

(12) McKay, S. L.; Haptonstall, B.; Gellman, S. H. *J. Am. Chem. Soc.* **2001**, *123*, 1244−1245.

(13) For other examples of aryl electronic control of protein stability, see: (a) Shi, Z. S.; Olson, C. A.; Kallenbach, N. R. *J. Am. Chem. Soc.* **2002**, *124*, 3284−3291. (b) Butterfield, S. M.; Patel, P. R.; Waters, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 9751−9755. (c) Tatko, C. D.; Waters, M. L. *J. Am. Chem. Soc.* **2002**, *124*, 9372−9373. (d) Waters, M. L. *Biopolymers* **2004**, *76*, 435−445.

(14) (a) Bretscher, L. E.; Jenkins, C. L.; Taylor, K. M.; DeRider, M. L.; Raines, R. T. *J. Am. Chem. Soc.* **2001**, *123*, 777−778. (b) Renner, C.; Alefelder, S.; Bae, J. H.; Budisa, N.; Huber, R.; Moroder, L. *Angew. Chem., Int. Ed.* **2001**, *40*, 923−925. (c) DeRider, M. L.; Wilkens, S. J.; Waddell, M. J.; Bretscher, L. E.; Weinhold, F.; Raines, R. T.; Markley, J. L. *J. Am. Chem. Soc.* **2002**, *124*, 2497−2505. (d) Taylor, C. M.; Hardre, R.; Edwards, P. J. B. *J. Org. Chem.* **2005**, *70*, 1306−1315. (e) Peptides containing 4*S*-fluoroproline (flp) were synthesized using proline editing: Thomas, K. M.; Naduthambi, D.; Tririya, G.; Zondlo, N. J. *Org. Lett.* **2005**, *7*, 2397−2400. (f) Relative to proline, 4*R*-fluoroproline (Flp) and 4*R*-hydroxyproline (Hyp) stabilize trans amide bonds, while 4*S*-fluoroproline (flp) destabilizes trans amide bonds.

(15) Vuister, G. W.; Bax, A. *J. Am. Chem. Soc.* **1993**, *115*, 7772−7777.

(16) Yao, J.; Dyson, H. J.; Wright, P. E. *J. Mol. Biol.* **1994**, *243*, 754−766.

(17) Che, Y.; Marshall, G. R. *J. Org. Chem.* **2004**, *69*, 9030−9042.

(18) See the Supporting Information for details.

(19) Vuister, G. W.; Delaglio, F.; Bax, A. *J. Am. Chem. Soc.* **1992**, *114*, 9674−9675.

(20) T(4-NH_2−Phe)flpN and TYflpN also exhibited small $^3J_{\alpha N}$ and upfield-shifted δ for cis-proline resonances, suggesting that electronic and stereoelectronic control may be a general approach to target type VI β-turn-mediated molecular recognition.

(21) Eberhardt, E. S.; Loh, S. N.; Raines, R. T. *Tetrahedron Lett.* **1993**, *34*, 3055−3056.

(22) Protein−DNA, protein−RNA and protein−small molecule complexes are also stabilized by proline−aromatic interactions. For example, the interactions of (electron-rich) polyphenols with proteins are mediated by proline-rich sequences: Charlton, A. J.; Haslam, E.; Williamson, M. P. *J. Am. Chem. Soc.* **2002**, *124*, 9899−9905.

JA057901Y