Amino Acids (2011) 41:181–186
DOI 10.1007/s00726-010-0504-8

ORIGINAL ARTICLE

Quantum mechanical origin of the conformational preferences of 4-thiaproline and its *S*-oxides

Amit Choudhary · Khian Hong Pua · Ronald T. Raines

Received: 24 November 2009 / Accepted: 25 January 2010 / Published online: 12 March 2010

Abstract The saturated ring and secondary amine of proline spawn equilibria between pyrrolidine ring puckers as well as peptide bond isomers. These conformational equilibria can be modulated by alterations to the chemical architecture of proline. For example, C^{γ} in the pyrrolidine ring can be replaced with sulfur, which can be oxidized either stereoselectively to yield diastereomeric *S*-oxides or completely to yield a sulfone. Here, the thiazolidine ring and peptide bond conformations of 4-thiaproline and its *S*-oxides were analyzed in an Ac–Xaa–OMe system using NMR spectroscopy, X-ray crystallography, and hybrid density functional theory. The results indicate that the ring pucker of the *S*-oxides is governed by the *gauche* effect, and the prolyl peptide bond conformation is determined by the strength of the $n \rightarrow \pi^*$ interaction between the amide oxygen and the ester carbonyl group. These findings, which are consistent with those of isologous 4-hydroxyprolines and 4-fluoroprolines, substantiate the importance of electron delocalization in amino acid conformation.

Keywords Collagen · $n \rightarrow \pi^*$ interaction · Stereoelectronic effect · Thiazolidine

A. Choudhary
Graduate Program in Biophysics,
University of Wisconsin-Madison,
1525 Linden Drive, Madison, WI 53706, USA

K. H. Pua · R. T. Raines
Department of Chemistry, University of Wisconsin-Madison,
1101 University Avenue, Madison, WI 53706, USA

R. T. Raines (✉)
Department of Biochemistry, University of Wisconsin-Madison,
433 Babcock Drive, Madison, WI 53706, USA
e-mail: rtraines@wisc.edu

Introduction

Proline is unique amongst the proteinogenic amino acids. Only proline has a saturated ring, and only proline is a secondary amine (Fischer 1906). These attributes give rise to two equilibria (Fig. 1). First, the pyrrolidine ring adopts a pucker in which C^{γ} is out-of-plane of the other ring atoms but flips between exo and endo conformers. Second, the prolyl peptide bond has a nearly equal preference for its *trans* (*Z*) and *cis* (*E*) isomers, and interconverts readily between these states.

In proline residues, the C^{γ}-endo ring pucker of proline residues is favored marginally over the C^{γ}-exo ring pucker (Improta et al. 2001; DeRider et al. 2002). Interestingly, substitutions at C^{γ} modulate the ring pucker preference. For example, replacement of the *pro-R* hydrogen with an electron-withdrawing group changes the preferred ring pucker from endo to exo. In contrast, an analogous replacement of the *pro-S* hydrogen amplifies the preference for the endo ring pucker over the exo ring pucker. These ring pucker preferences have been explained on the basis of the *gauche* effect (Eberhardt et al. 1996), which arises when two vicinal atoms bear electronegative substituents. These substituents prefer to reside gauche to each other (i.e. with a dihedral angle of $\pm 60°$) to maximize the overlap between their σ^* orbitals and σ orbitals involving more electropositive substituents.

Other substitutions enable modulation of the conformational properties of proline (Kern et al. 1997; Holmgren et al. 1998; Wittelsberger et al. 2000; Zhu et al. 2002; Melis et al. 2009). (2*R*)-4-Thiaproline (Thp) is an especially interesting analog in which sulfur replaces the out-of-plane C^{γ} of the pyrrolidine ring. Recently, we reported that this analog is a substrate for human prolyl 4-hydroxylase (P4H), the enzyme that catalyzes the post-translational

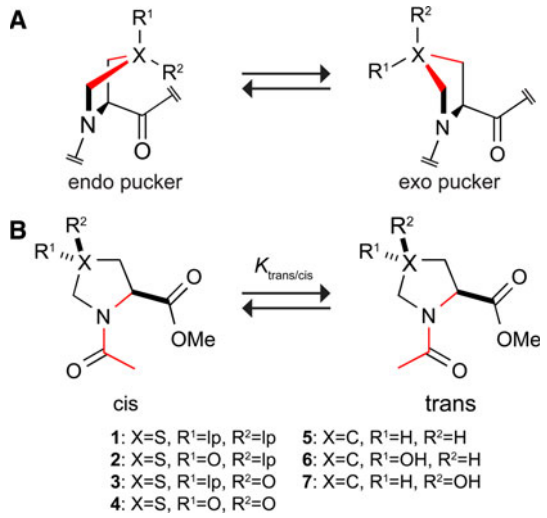

Fig. 1 Conformations of proline-like residues. **a** Pyrrolidine ring pucker, **b** amide-bond isomerization state. *lp* lone pair

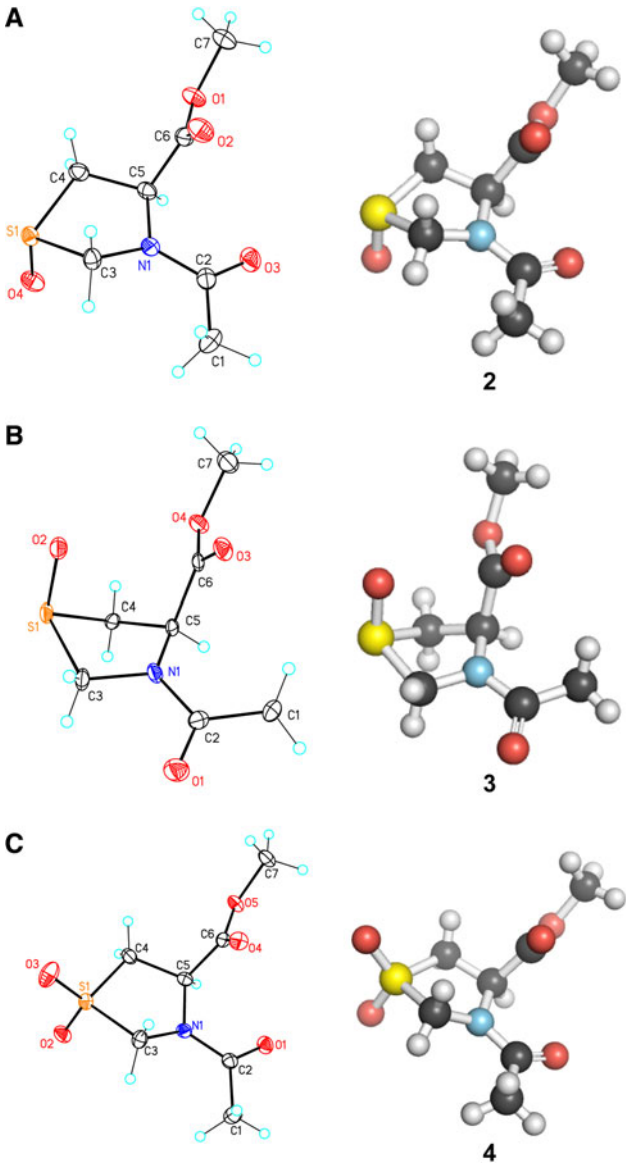

Fig. 2 Crystal structures of compounds **a 2**, **b 3**, and **c 4**. *Left* thermal ellipsoids, *right* ball-and-stick representations

hydroxylation of proline residues in protocollagen strands (Gorres et al. 2008). In a peptidic substrate, P4H oxidizes Thp to its *S*-oxide stereoselectively.

The electronic structure of a thiazolidine ring differs from that of a pyrrolidine ring, and its ring conformations could be stabilized by different donor–acceptor orbital interactions. Moreover, stereoselective oxidation of sulfur in Thp can yield one of the two diastereomeric *S*-oxides (**2** and **3**; Fig. 2). This chemical transformation is analogous to the installation of an electron-withdrawing group at the C^{γ} of the pyrrolidine ring. Although low-resolution structures of **1**–**3** are available (Goodman et al. 1970a, b; Nachtergaele and Anteunis 1980), we sought to determine the atomic resolution structures of **1**–**3** using X-ray crystallography and/or hybrid density functional theory (DFT). Such high-resolution picture is indispensable for deciphering the various donor–acceptor orbital interactions that dictate the ring pucker preferences and accurately mapping the structure of P4H active site.

In a Xaa-Pro peptidic system, a well-known correlation exists between the pyrrolidine ring pucker and the *cis*–*trans* equilibrium of the Xaa-Pro amide bond (Fig. 1) (Milner-White et al. 1992). The stabilization of the exo ring pucker over the endo ring pucker increases the population of the *trans* conformation, while the stabilization of the endo ring pucker over the exo ring pucker increases the population of the *cis* conformation. In addition to characterizing thiazolidine ring pucker, we searched for a correlation between that ring pucker and the isomerization state of the amide bond.

The Ac–Xaa–OMe model system provides an accurate and simple way to determine the conformational preferences of proline and its analogs (Fig. 1). The use of a methyl ester instead of an amide obviates the intramolecular hydrogen bonding that has been observed in Ac-Pro-NHMe (Matsuzaki and Iitaka 1971; Higashijima et al. 1977; Liang et al. 1992; Benzi et al. 2002). Accordingly, we synthesized Ac-Thp-OMe (**1**) and its two *S*-oxides (**2**, **3**). Herein, we report the structure and the conformational preferences of **1**–**3** using NMR spectroscopy, X-ray crystallography, and ab initio calculations. Our findings inform a wide spectrum of activities in protein chemistry as well as medicinal chemistry, as thiazoldine ring is a privileged scaffold in drug design (Prabhakar et al. 2006).

Materials and methods

Synthesis

Compounds **1**–**4** were synthesized using procedures reported previously (Nachtergaele and Anteunis 1980; Aitken et al. 1997).

Computational analyses

Geometry optimization and frequency calculations were carried on four conformations for each model compound (**1**–**4**) at the B3LYP/6-311+G(2d,p) level of theory with Gaussian '03 (Gaussian 03). The frequency calculations indicated that these conformations were indeed true stationary points on the potential energy surface. Each of these optimized geometries was then analyzed with NBO 5.0 at B3LYP/6-311+G(2d,p) level of theory to determine the contribution of specific donor–acceptor orbital interaction to conformational stability.

X-ray crystallography

The desired compounds were dissolved in hexanes with a minimal amount of EtOAc. Slow evaporation afforded crystals suitable for X-ray analysis after ~2 weeks. X-ray intensity data were collected on a Bruker CCD-1000 diffractometer with Mo K_α ($\lambda = 0.71073$ Å) radiation at 105(2) K with the diffractometer to crystal distance of 4.9 cm. Preliminary indexing was carried out to determine the cell constants. This indexing consisted of three series of ω scans at different initial angles with each series consisting of 20 frames at intervals of 0.3° with an exposure time of 10 s per frame. The reflections were indexed using an automated indexing routine built in the SMART program. Data were collected with the full-sphere data collection routine to a resolution of 0.80 Å. The intensity data were then corrected for absorption and Lorentz and polarization effects. Structure solution and refinement were carried out using SHELXTL V.6.10 (Bruker-AXS 2000-2003).

NMR spectroscopy

For the measurement of $K_{trans/cis}$ values, each compound (5–10 mg) was dissolved in D_2O with enough CD_3OD added to solubilize the compound (<20% of total volume). ^1H spectra were acquired and analyzed with the software package NUTS (Acorn NMR). The values of $K_{trans/cis}$ were determined from the relative areas of the *trans* and *cis* peaks. NOEDIFF experiments were carried out to confirm the proton assignments.

Results and discussion

The structures of **1**–**3** were determined at atomic resolution with hybrid density functional theory (**1**–**3**) and X-ray crystallography (**2**, **3**; Fig. 2). The atomic coordinates of compounds **1**–**4** are listed in Tables S2, S4, S10, and S16, respectively, of the supporting information. After determining the structure of Ac-Thp-OMe (**1**) and its *S*-oxides (**2** and **3**) at atomic resolution, we compared their ring parameters with those of the analogous proline derivatives: Ac-Pro-OMe (**5**), Ac-Hyp-OMe (**6**) (Panasik et al. 1994), and Ac-hyp-OMe (**7**) (Shoulders 2009) (Table 1; Fig. 3a, b), where "Hyp" refers to (2*S*,4*R*)-4-hydroxyproline and "hyp" refers to (2*S*,4*S*)-4-hydroxyproline. Compound **2**, like **6**, adopts the exo ring pucker, whereas compound **3,** like **7**, adopts the endo ring pucker. The ring torsion angles of the *S*-oxides and the hydroxyprolines are similar. The only notable differences include a larger ring for the *S*-oxides compared to that of the hydroxyprolines, and a larger ϕ angle for **2**. Hence, we put forth the 4*R* diastereomer of Thp *S*-oxide as a surrogate for Hyp, which is the product of the most prevalent post-translational modification in animals (Gorres and Raines 2010).

Table 1 Ring parameters for compounds **1–7**

Torsion angle	Compound							
	1[a]	**2**[b]	**3**[b]	**4**[b]	**5**[b]	**6**[b,c]	**6**[b,c]	**7**[b]
C^δ–X–C^β–C^α	–20.62	30.66	–43.12	15.18	–37.22	36.36	40.21	–38.27
N–C^δ–X–C^β	–1.73	–39.23	31.37	–28.16	25.18	–30.41	–32.87	23.59
C^α–N–C^δ–X	26.46	39.51	–10.71	35.58	–3.24	13.59	13.77	0.31
C^β–C^α–N–C^δ	–43.13	–16.60	–20.75	–25.84	–19.69	8.81	10.70	–23.99
X–C^β–C^α–N	37.90	–13.89	43.02	2.46	34.51	–27.67	–31.12	37.77

[a] From DFT calculations

[b] From X-ray diffraction analysis of the crystalline compounds

[c] There are two independent molecules in the unit cell

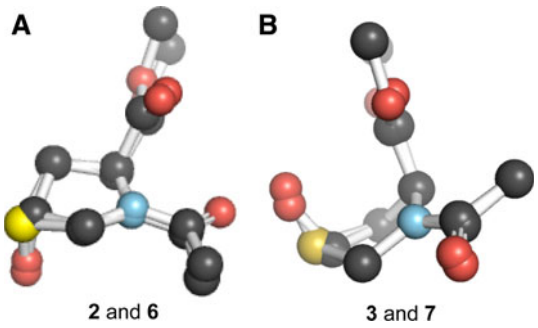

Fig. 3 Overlay of isologous crystal structures. **a** Compounds **2** and **6**, which are in the C^{γ}-exo conformation. **b** Compounds **3** and **7,** which are in the C^{γ}-endo conformation

Hybrid density functional theory (DFT) and natural bond orbital (NBO) analyses (Weinhold 1998; Glendening et al. 2001; Weinhold and Landis 2005) were used to determine the donor–acceptor orbital interactions that dictate the ring pucker preferences of compounds **1**–**3**. These computational analyses indicate that the most stable conformation in the gas phase for **1** is *trans* endo, whereas for **2** is *trans* exo. In the exo conformation of **2**, the antibonding orbital of the S–O bond is positioned for an extensive overlap with the $\sigma(C^{\beta}–H)$ and $\sigma(C^{\delta}–H)$ bonding orbitals (Fig. 4a). This extensive overlap of the orbitals cannot be attained in the endo conformation of **2**. Similar

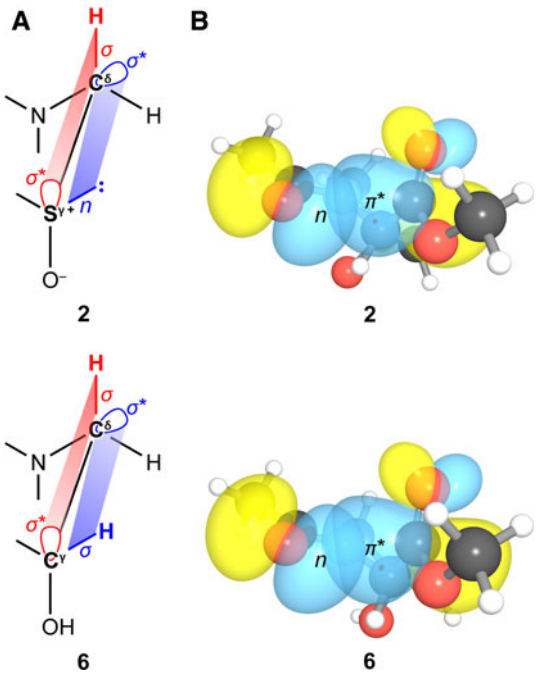

Fig. 4 Quantum mechanical origin of the stereoelectronic effects in the preferred conformation of compounds **2** and **6**. **a** Overlap of σ (or n) and σ^* orbitals that gives rise to the *gauche* effect. **b** Overlap of the lone pair and π^* orbital that gives rise to the $n \rightarrow \pi^*$ interaction; depictions were generated with NBOView 1.1 (Wendt and Weinhold 2001)

electronic delocalization takes place in the endo conformation of **3** and cannot occur in its exo conformation. These electronic delocalizations are the primary cause for the stabilization of the exo and endo conformations for **2** and **3**. Analogous electronic delocalization has been reported before (Tshuchishashi et al. 1973; Ulshöfer and Podlech 2009).

A strong correlation exists between the ring pucker and the $K_{trans/cis}$ (Scheme 1). This correlation is due to an $n \rightarrow \pi^*$ interaction in which a lone pair (n) of an amide oxygen (O_{i-1}) overlaps with the antibonding orbital (π^*) of $C_i = O_i$ of the subsequent amide (Fig. 4b) (DeRider et al. 2002; Hinderaker and Raines 2003). Such an interaction differentially stabilizes the *trans* conformation over the *cis* conformation, as it occurs in the former but not the latter. In the exo pucker, the donor oxygen and the acceptor carbonyl group are closer than in the endo pucker. Thus, compounds with a high population of the exo ring pucker have a stronger $n \rightarrow \pi^*$ interaction and exhibit a larger $K_{trans/cis}$ value. The preference for the exo conformation decreases in the order **2** > **1** > **3**, which is parallel to the decrease in the value of $K_{trans/cis}$ (Table 2).

Compound **4** offers an opportunity to test our hypothesis regarding the basis for the correlation of ring pucker and $K_{trans/cis}$ (Scheme 1). The sulfur in compound **2** can be oxidized further to form a sulfone. The antibonding orbital of the new S–O bond is positioned to stabilize the endo conformation of **4**. An increase in the population of the endo conformation should lower the value of $K_{trans/cis}$. The $K_{trans/cis}$ of **4** is indeed lower than that of **2** and equal to that of **1** (Table 2). This finding aligns compounds **2**–**4** with analogous 4-fluoroprolines, in which the 4*R* diastereomer has a high $K_{trans/cis}$ value, the 4*S* diastereomer has a low value, and the unsubstituted and doubly substituted derivatives have equivalent values that are intermediate (Shoulders et al. 2009). These $K_{trans/cis}$ values for eight compounds in two distinct classes (Fig. 1: X = S, R^1 = lp or O, R^2 = lp or O; X = C, R^1 = H or F, R^2 = H or F) are consistent with Scheme 1 and its underlying quantum mechanical origin.

The crystal structure of compound **3** offers additional insight into the nature of the forces that dictate the conformational preferences of these molecules. In crystal structures, carbonyl groups often form short contacts with electron-pair donor groups in a manner reminiscent of the nucleophilic attack along the Bürgi–Dunitz trajectory. The origin of these short contacts has been attributed to dipole–dipole interactions (Paulini et al. 2005; Fischer et al. 2008).

Scheme 1 Correlation between ring pucker and $K_{trans/cis}$ in proline and its analogs

Table 2 Conformational parameters for compounds **1–4**

Compound	Conformational data					
	$K^{a}_{trans/cis}$	Ring pucker[b]	d (Å)[b]	θ (°)[b]	Δ (Å)[b]	Θ (°)[b]
1	2.8	Endo	ND	ND	ND	ND
2	4.0	Exo	3.01	91.9	0.025	2.88
3	1.6	Endo	ND	ND	–0.054	–6.28
4	2.8	Exo	2.99	94.8	0.018	2.14

[a] In D_2O at 25°C; values are ± 10%

[b] From X-ray diffraction analysis of the crystalline compound

d is the $O_{i-1}\cdots C_i'$ distance; θ is the $O_{i-1}\cdots C_i'{=}O_i'$ angle; Δ is the $C_i'\cdots$plane($C_i^{\alpha}\ O_i'\ O_{i+1}$) distance; and Θ is the $C_i'{=}O_i'\cdots$plane ($C_i^{\alpha}\ C_i'\ O_{i+1}$) angle (Choudhary et al. 2009). *ND* not determined

Recently, we reported that such short contacts arise instead from $n \rightarrow \pi^*$ electronic delocalization (Choudhary and Raines 2009; Choudhary et al. 2009). In the crystal structure of **3**, a short contact exists between the sulfoxide oxygen and the ester carbonyl group. This short contact cannot arise from a dipole–dipole interaction, and the two interacting dipoles can be in a repulsive orientation. One can, however, envision an $n \rightarrow \pi^*$ electronic delocalization from a lone pair (n) of the sulfoxide oxygen into the antibonding orbital (π^*) of the ester carbonyl group (Lesarri et al. 2005). This explanation is consistent with the observed pyramidalization of the carbonyl carbon (Table 2), which is an unambiguous indication of covalency (Choudhary and Raines 2009; Choudhary et al. 2009).

Conclusions

We have examined the conformational preferences of compounds **1–4** using NMR spectroscopy, X-ray crystallography, and ab initio calculations. Our data indicate that whereas, **1**, **3**, and **4** prefer the endo conformation and have a low value of $K_{trans/cis}$, **2** prefers the exo conformation and has a high value of $K_{trans/cis}$. These findings on thiazolidine rings are in accord with previous findings on pyrrolidine rings (Scheme 1). The conformational preferences in both ring systems have a tractable quantum mechanical origin.

Acknowledgments We thank C. N. Bradford, B. R. Caes, G. A. Ellis, and K. L. Gorres for contributive discussions. This work was supported by grant R01 AR044276 (NIH). K.H.P. was supported by a Mary Shine Peterson award.

References

Aitken RA, Mesher STE, Ross FC, Ryan BM (1997) Effect of added benzoic acid on the phase-transfer catalysed permanganate oxidation of organosulfur compounds. Synthesis 7:787–791

Benzi C, Improta R, Scalmani G, Barone V (2002) Quantum mechanical study of the conformational behavior of proline and 4*R*-hydroxyproline dipeptide analogues in vacuum and in aqueous solution. J Comput Chem 23:341–350

Choudhary A, Raines RT (2009) A donor–acceptor perspective on carbonyl–carbonyl interactions in proteins. In: Lebl M (ed) Breaking away: the proceedings of the twenty-first American peptide symposium. Prompt Scientific Publishing, San Diego, CA, pp 347–349

Choudhary A, Gandla D, Krow GR, Raines RT (2009) Nature of amide carbonyl–carbonyl interactions in proteins. J Am Chem Soc 131:7244–7246

DeRider ML, Wilkens SJ, Waddell MJ, Bretscher LE, Weinhold F, Raines RT, Markley JL (2002) Collagen stability: insights from NMR spectroscopic and hybrid density functional computational investigations of the effect of electronegative substituents on prolyl ring conformations. J Am Chem Soc 124:2497–2505

Eberhardt ES, Panasik N Jr, Raines RT (1996) Inductive effects on the energetics of prolyl peptide bond isomerization: implications for collagen folding and stability. J Am Chem Soc 118:12261–12266

Fischer E (1906) Untersuchungen über Aminosaüren, Polypeptide und Proteine. Ber Deutsch Chem Ges 39:530–610

Fischer FR, Wood PA, Allen FH, Diederich F (2008) Orthogonal dipolar interactions between amide carbonyl groups. Proc Natl Acad Sci USA 105:17290–17294

Glendening ED, Badenhoop JK, Reed AE, Carpenter JE, Bohmann JA, Morales CM, Weinhold F (2001) NBO 5.0. Theoretical Chemistry Institute. University of Wisconsin-Madison, Madison, WI

Goodman M, Niu GC-C, Su K-c (1970a) Conformational aspects of polypeptide structure. XXXII. Helical poly[(*S*)-thiazolidine-4-carboxylic acid]. Theoretical results. J Am Chem Soc 92:5219–5220

Goodman M, Su K-c, Niu GC-C (1970b) Conformational aspects of polypeptide structure. XXXII. Helical poly[(*S*)-thiazolidine-4-carboxylic acid]. Experimental results. J Am Chem Soc 92:5220–5222

Gorres KL, Raines RT (2010) Prolyl 4-hydroxylase. Crit Rev Biochem Mol Biol 45:106–124

Gorres KL, Edupuganti R, Krow GR, Raines RT (2008) Conformational preferences of substrates for human prolyl 4-hydroxylase. Biochemistry 47:9447–9855

Higashijima T, Tasumi M, Miyazawa T (1977) ^{1}H Nuclear magnetic resonance studies of *N*-acetyl-L-proline *N*-methylamide. Molecular conformations, hydrogen bondings, and thermodynamic quantities in various solvents. Biopolymers 16:1259–1270

Hinderaker MP, Raines RT (2003) An electronic effect on protein structure. Protein Sci 12:1188–1194

Holmgren SK, Taylor KM, Bretscher LE, Raines RT (1998) Code for collagen's stability deciphered. Nature 392:666–667

Improta R, Benzi C, Barone V (2001) Understanding the role of stereoelectronic effects in determining collagen stability. 1. A quantum mechanical study of proline, hydroxyproline, and fluoroproline dipeptide analogues in aqueous solution. J Am Chem Soc 123:12568–12577

Kern D, Schutkowski M, Drakenberg T (1997) Rotational barriers of *cis*/*trans* isomerization of proline analogues and their catalysis by cyclophilin. J Am Chem Soc 119:8403–8408

Lesarri A, Cocinero EJ, López JC, Alonso JL (2005) Shape of 4*S*- and 4*R*-hydroxyproline in gas phase. J Am Chem Soc 127:2572–2579

Liang G-B, Rito CJ, Gellman SH (1992) Variations in the turn-forming characteristics of *N*-acyl proline units. Biopolymers 32:293–301

Matsuzaki T, Iitaka Y (1971) The crystal structure of acetyl-L-proline-*N*-methylamide. Acta Crystallogr B27:507–516

Melis C, Bussi G, Lummis SCR, Molteni C (2009) *Trans–cis* switching mechanisms in proline analogues and their relevance for the gating of the 5-HT_3 receptor. J Phys Chem B 113:12148–12153

Milner-White JE, Bell LH, Maccallum PH (1992) Pyrrolidine ring puckering in *cis* and *trans*-proline residues in proteins and polypeptides. J Mol Biol 228:725–734

Nachtergaele WA, Anteunis MJO (1980) Ring conformational aspects of cis- and *trans-N*-acetyl-4-carbomethoxy-1,3-thiazolidine-1-oxide. Bull Soc Chim Belg 89:749–758

Panasik N Jr, Eberhardt ES, Edison AS, Powell DR, Raines RT (1994) Inductive effects on the structure of proline residues. Int J Pept Protein Res 44:262–269

Paulini R, Müller K, Diederich F (2005) Orthogonal multipolar interactions in structural chemistry and biology. Angew Chem Int Ed 44:1788–1805

Prabhakar YS, Solomon VR, Gupta MK, Katti SB (2006) QSAR studies on thiazolidines: a biologically priviliged scaffold. Top Heterocycl Chem 4:161–249

Shoulders MD (2009) Ph.D. thesis, University of Wisconsin-Madison

Shoulders MD, Kamer KJ, Raines RT (2009) Origin of the stability conferred upon collagen by fluorination. Bioorg Med Chem Lett 19:3859–3962

Tshuchishashi G-i, Mitamura S, Inoue S, Ogura K (1973) Asymmetric synthesis using α,β-unsaturated sulfoxides. A highly selective Michael reaction. Tetrahedron Lett 323-326

Ulshöfer R, Podlech J (2009) Stereoelectronic effects in vinyl sulfoxides. J Am Chem Soc 131:16618–16619

Weinhold F (1998) Natural bond orbital methods. In: PvR Schleyer, Allinger NL, Clark T, Gasteiger J, Kollman PA, Shaefer HF III et al (eds) Encyclopedia of computational chemistry, vol 3. John Wiley & Sons, Chichester, UK, pp 1792–1811

Weinhold F, Landis CR (2005) Valency and bonding: a natural bond orbital donor–acceptor perspective. Cambridge University Press, Cambridge, UK

Wendt M, Weinhold F (2001) NBOView 1.1. Theoretical Chemistry Institute. University of Wisconsin-Madison, Madison WI

Wittelsberger A, Keller M, Scarpellino L, Patiny L, Orbea-Acha H, Mutter M (2000) Pseudoprolines:targeting a *cis* conformation in a mimetic of the gp120 V3 loop of HIV-1. Angew Chem Int Ed 39:1111–1115

Zhu W, Gincherman Y, Docherty P, Spilling CD, Becker DF (2002) Effects of proline analog binding on the spectroscopic and redox properties of PutA. Arch Biochem Biophys 408:131–136