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ORIGINAL ARTICLE

Impacts of terminal (4*R*)-fluoroproline and (4*S*)-fluoroproline residues on polyproline conformation

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Abstract Many interests have been focused on prolyl *cis–trans* isomerization which is related to protein folding and isomer-specific biochemical recognition. Since polyproline can adopt either type I (PPI) helices with all *cis* amide bonds or type II (PPII) helices with all *trans* amide bonds, it has been a valuable model to study the prolyl isomerization. Recent studies have shown that stereoelectronic effects govern the stability of PPII structure and the rate of PPII → PPI conversion. To further explore the terminal stereoelectronic effects on polyproline conformation, herein we synthesized a series of host–guest peptides in which (2*S*,4*S*)-4-fluoroproline (flp) or (2*S*,4*R*)-4-fluoroproline (Flp) residues are incorporated into the C- or N-terminal end of a peptide and studied the thermodynamic and kinetic consequences on polyproline conformation. Circular dichroism measurements revealed that inserting 4-fluoroproline residues into the C terminus of a polyproline peptide induces a great stereoelectronic effect on PPII stability and PPII → PPI conversion rates. From the C terminus, a $(Flp)_3$ triplet stabilizes PPII structure and increases the transition barrier of PPII → PPI conversion by 1.53 kJ mol^{-1} while a $(flp)_3$ triplet destabilizes PPII conformation and reduce the PPII → PPI transition barrier by 4.61 kJ mol^{-1}. In contrast, the 4-fluoroproline substitutions at the N terminus do not exhibit distinct stereoelectronic effects on PPII stability and PPII → PPI conversion rates. Our data demonstrate that the C-terminal stereoelectronic effects have a more dramatic impact on PPII stability and PPII → PPI conversion kinetics.

Keywords Stereoelectronic effect · Polyproline · 4-fluoroproline · Prolyl isomerization · Transition state barrier

Introduction

Proline has a lower energy gap than other amino acids in the isomerization of ω-dihedral angles in proteins due to a comparable steric situation between *cis* and *trans* conformations. Prolyl isomerization has been frequently found in the folding of proteins (Ferreon and Hilser 2003; Hamburger et al. 2004; Shi et al. 2006; Wedemeyer et al. 2002; Whittington et al. 2005) and the biological effects related to isomer-specific recognition (Brandsch et al. 1998; Kleywegt and Jones 1996; Sarkar et al. 2007; Siligardi and Drake 1995; Vanhoof et al. 1995). Due to the structural constrains of five-membered ring and no hydrogen bond donating capacity, proline also plays an important role in the secondary structure of globular proteins (Adzhubei and Sternberg 1993; Cubellis et al. 2005; Stapley and Creamer 1999). Polyproline can form either type I (PPI) or type II (PPII) helices. PPI is a right-handed helix containing all *cis* amide bonds ($\omega = 0°$) and adopts backbone dihedral angles of ($\phi = -75°$, $\psi = 160°$). In contrast, PPII is a left-handed helix containing all *trans* amide bonds ($\omega = 180°$) and adopts backbone dihedral angles of ($\phi = -75°$, $\psi = 145°$). PPI structure is more compact than PPII

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structure, and is favored in specific organic solvents, such as *n*-propanol and butanol, whereas the PPII helix dominates in aqueous solution (Adzhubei and Sternberg 1993; Cubellis et al. 2005; Kakinoki et al. 2005; Knof and Engel 1974; Mutter et al. 1999; Stapley and Creamer 1999). In nature, proline-rich sequences in proteins often form PPII helices, which play important roles in structure and function (Adzhubei et al. 2013) and are often regarded as the predominated structures in unfolded proteins (Mezei et al. 2004; Shi et al. 2002, 2006). Since polyproline can adopt either all *trans* or all *cis* peptide bond helices, it has been a valuable model for studying the *cis–trans* isomerizations of amide bonds (Dugave and Demange 2003; Fischer 2000).

In the literature, a few studies reported the use of NMR and UV–Vis spectroscopy to investigate the prolyl *cis–trans* isomerization kinetics in short peptides or proteins (Fischer 2000; Reimer et al. 1998; Steinberg et al. 1960). Brandts and co-workers also utilized aminopeptidase P that can cleave the *trans* prolyl bond at the N-terminal end of a peptide to study the prolyl isomerization in oligopeptides, and demonstrated that the rate of hydrolysis for prolyl bonds is consistent with the prolyl *cis–trans* isomerization kinetics (Lin and Brandts 1979). Following that work, they further studied the isomerization for a polyproline helix and found that the rate of all *cis*-prolyl bonds undergoing hydrolysis was nearly identical to the *cis* to *trans* conversion rate measured by circular dichroism (CD), which led to the conclusion that the *cis* to *trans* isomerization begins at the N terminus whereas the *trans* to *cis* isomerization initiates at the C terminus for a polyproline helix (Lin and Brandts 1980).

Although the pyrrolidine ring of proline can adopt a C^{γ}-*exo* or a C^{γ}-*endo* pucker, the puckering preference may be modulated by installation of electron-withdrawing substituents (e.g., OH, F) into the 4*R* or 4*S* position, and the stereoelectronic effects from such 4-substituted proline derivatives have been applied to examine the origin of collagen stability in the past decade (Shoulders and Raines 2009). Raines and co-workers also demonstrated that the proline derivatives with an electron-withdrawing group on the 4*R* position and a biased C^{γ}-*exo* pucker would form strong backbone $n \rightarrow \pi^*$ interactions to favor *trans* amide bonds and enhance PPII stability (Horng and Raines 2006; Hinderaker and Raines 2003). It was known that a polyproline helix would proceed *cis* to *trans* or *trans* to *cis* isomerization actually depending on the solvent condition (Kakinoki et al. 2005; Knof and Engel 1974; Mutter et al. 1999), and thus the interconversion rates between PPI and PPII helices could be measured by switching solvent conditions. Recently, we have used 4-substituted proline derivatives and solvent-induced conformational changes to examine stereoelectronic effects on the kinetics of polyproline conformational interconversion (Chiang et al. 2009). In the study, we found that stereoelectronic effects have a significant impact on the transition energy barrier of PPII → PPI conversion, and 4-fluoro-proline derivatives in particular have the most pronounced effects.

Even though the *cis–trans* isomerization mechanism of prolyl peptide bonds and stereoelectronic effects on the PPI ↔ PPII interconversion rates have been studied, more detailed kinetic information needs to be unraveled. In addition, the terminal functional groups were shown to affect PPII stability (Kuemin et al. 2009), suggesting that stereoelectronic effects on polyproline conformation may be position dependent. Accordingly, we studied terminal stereoelectronic effects on the stability of PPII structure and the transition barrier of PPI ↔ PPII interconversion in this work. To pursue such a task, here we synthesized a series of peptides in which multiple (2*S*, 4*R*)-4-fluoroproline (Flp) or (2*S*, 4*S*)-4-fluoroproline (flp) residues are incorporated into the N-terminal or C-terminal end of a polyproline peptide, and studied the thermodynamic and kinetic consequences on polyproline structure by CD measurements. From experimental data, we find that C-terminal stereoelectronic effects can govern the stability of PPII structure and the kinetics of PPII → PPI conversion while N-terminal stereoelectronic effects in contrast have only a small impact on PPII stability and PPII → PPI conversion rates. Furthermore, the kinetics of PPI → PPII conversion is not strongly correlated with terminal stereoeletcronic effects. The significant impact of C-terminal stereoelectronic effects on the conversion of PPII → PPI also concurs with the C to N mechanism of *trans–cis* isomerization for prolyl peptide bonds.

Experimental procedures

General

Reagents were obtained from Aldrich-Sigma Chemical, Alfa Aesar, Fluka, or Novabiochem, and used without further purification. Amino acids, *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU), and hydroxybenzotriazole (HOBt) were obtained from Advanced ChemTech or AnaSpec, and Fmoc-(2*S*,4*R*)-4-fluoroproline was purchased from Bachem Bioscience. Fmoc-(2*S*,4*S*)-4-fluoroproline was synthesized as described previously (Horng and Raines 2006). MALDI-TOF mass spectra of the peptides were obtained using an Autoflex III Smartbeam LRF200-CID spectrometer (Bruker Daltonics).

Attachment of Fmoc-Pro-OH, Fmoc-Flp-OH, and Fmoc-flp-OH to 2-chlorotrityl resin

Under N_2(g), 0.20 mmol of 2-chlorotrityl resin (200 mg, loading 1.0 mmol g^{-1}) were swelled in dry CH_2Cl_2

(3.0 mL). A solution of Fmoc-protected amino acid (0.20 mmol) and DIEA (0.13 mL, 0.60 mmol) in dry CH_2Cl_2 (1.5 mL) was added by syringe. An additional 2.0 mL of dry CH_2Cl_2 was used to ensure complete transfer. After 2 h, 2.5 mL of anhydrous CH_3OH was added to the mixture to cap any remaining active sites on the resin. The resin-bound peptide was isolated by gravity filtration, washed with several portion of dry CH_2Cl_2 ($\sim$ 30 mL), and dried at reduced pressure. Loading was measured by UV spectroscopy using the reported protocol (Applied Biosystems Technical Note 123485, Rev. 2, http://www3.appliedbiosystems.com/sup/gl/search.htm) to be 0.77 mmol g^{-1} for Fmoc-Pro-resin, 0.55 mmol g^{-1} for Fmoc-Flp-resin, and 0.41 mmol g^{-1} for Fmoc-flp-resin, respectively.

Peptide synthesis and purification

A series of polyproline peptides as shown in Table 1 were prepared on a 0.05-mmol scale by standard solid-phase methods and Fmoc-chemistry protocols. Fmoc-protected amino acids were used and HBTU-mediated coupling reactions were carried out on an automated PS3 peptide synthesizer (Proteins Technologies Inc.).

Use of a 2-chlorotrityl resin that was pre-loaded with amino acid (as described above) generated a free C terminus upon cleavage from the resin with a solution of 95 % (v/v) trifluoroacetic acid (TFA), 2.5 % triisopropylsilane, and 2.5 % H_2O. Each peptide has a free N terminus. All peptides were purified by reverse-phase high-performance liquid chromatography (HPLC) with a Vydac semipreparative C18 column. H_2O/acetonitrile gradients with 0.1 % TFA as the counterion were used for the eluting solvent system. All purified peptides were more than 90 % pure according to HPLC analysis. The identities of all peptides were confirmed by MALDI-TOF mass spectrometry. The calculated and observed molecular weights are shown in Table 1.

Table 1 Sequences and molecular weights for the polyproline peptides used in this study

Peptide	Sequence[a]	Calculated [M]	Observed
P11	NH-$(Pro)_{11}$-OH	1085.6	$[MH^+]$ 1086.4
N-Flp-P11	NH-$(Flp)_3$-$(Pro)_8$-OH	1139.6	$[MK^+]$ 1178.9
C-Flp-P11	NH-$(Pro)_8$-$(Flp)_3$-OH	1139.6	$[MNa^+]$ 1162.7
N-flp-P11	NH-$(flp)_3$-$(Pro)_8$-OH	1139.6	$[MNa^+]$ 1162.8
C-flp-P11	NH-$(Pro)_8$-$(flp)_3$-OH	1139.6	$[MH^+]$ 1140.3

[a] Flp is (2*S*,4*R*)-4-fluoroproline, and flp is (2*S*,4*S*)-4-fluoroproline. Each peptide has a free N terminus and a free C terminus

Circular dichroism (CD) spectroscopy

All CD measurements were performed using an Aviv Model 410 circular dichroism spectrometer. Far-UV CD spectra were obtained at 4 °C in pH 7.0 and 20 mM sodium phosphate buffer or *n*-propanol using a 1-mm quartz cuvette and a spectrometer bandwidth of 1 nm. The peptide concentration was 50-150 μM. Due to the lack of aromatic residues in the peptides, the peptide concentrations were determined using the UV absorbance at 205 nm (Grimsley and Pace 2003; Scopes 1974). The time-dependent experiments were conducted at 4 °C for the samples in 90 % (v/v) aqueous solution or 95 % (v/v) *n*-propanol. All samples in *n*-propanol were incubated at 4 °C for more than 4 days before measurements to allow the formation of PPI helices.

Results and discussion

From our previous study, we used host–guest peptides to unravel that stereoelectronic effects play an important role in modulating the transition barrier of PPII → PPI conversion, suggesting that the kinetic consequences resulted from stereoelectronic effects might mediate PPII stability (Chiang et al. 2009). To learn about how stereoelectronic effects influence polyproline structure and conformational interconversion from different ends of a polyproline helix, we designed and synthesized a series of peptides (Table 1). In our design, we chose two 4-fluoro substituted proline derivatives, Flp and flp, to replace Pro in the peptide because we had found that 4-fluoroproline had the most profound effects on the transition barrier of PPII → PPI conversion (Chiang et al. 2009). Flp favors an *exo* C^γ-pucker facilitating the backbone $n \rightarrow \pi^*$ to stabilize a PPII helix while its diastereomer flp prefers an *endo* C^γ-pucker and the formation of a PPI helix, and thus their influences on polyproline can be compared. Moreover, to have a pronounced effect, we substituted three consecutive Pro residues with Flp or flp at the terminus of a peptide.

Terminal 4-fluoroproline residues on PPII stability

CD spectroscopy was used to characterize the peptide structure in solution. In aqueous solution, all the peptides exhibit a positive band between 220 and 230 nm and a negative band at 205 nm in their far-UV CD spectra (see Fig. S1 of the supplementary material), indicating that all the peptides form PPII structure. Since each peptide has a very similar CD spectrum, it is very difficult to compare their PPII stability solely based on the measurements. PPII helices dominate in aqueous solution and PPI helices are favored in some specific organic solvents, such as *n*-propanol, and thus we used the amount of *n*-propanol in

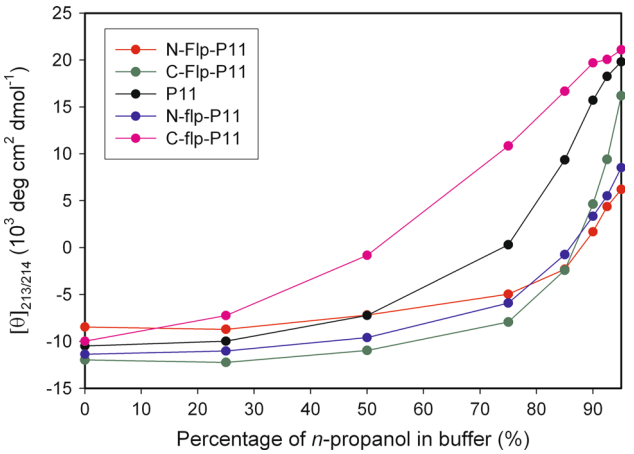

Fig. 1 Plots of molar ellipticity at 213 or 214 nm versus percentage of *n*-propanol in phosphate buffer for the peptides at 4 °C

aqueous solution required to switch PPII to PPI to evaluate the PPII stability for these peptides. Upon adding various fractions of *n*-propanol in phosphate buffer, the positive band gradually shifts to 213 or 214 nm in the far-UV CD spectra of the peptides, indicating the conversion of PPII to PPI structure (see Fig. S2 of the supplementary material). For comparing the propensity of converting PPII to PPI structure, we plotted the molar ellipticity at 213 or 214 nm against the percentage of *n*-propanol in phosphate buffer. As shown in Fig. 1, both N-Flp-P11 and N-flp-P11 exhibit a stronger resistance than P11 to convert their conformation from PPII to PPI, and no distinct tendencies can be drawn from these two peptides, indicating that N-terminal stereoelectronic effects may not be a critical factor affecting PPII stability. This result is somewhat surprising because Flp was previously shown to stabilize PPII helices via stereoelectronic effects while flp was found to destabilize PPII helices (Horng and Raines 2006). In contrast, C-Flp-P11 and C-flp-P11 display a totally different resistance to the conversion of PPII to PPI. Compared to P11, C-Flp-P11 is more persistent to PPII conformation while C-flp-P11 transforms to PPI structure more easily, suggesting that stereoelectronic effects imposed on the C terminus have a critical impact on PPII stability. These experimental data suggest that stereoelectronic effects on polyproline conformation may be directional.

Terminal 4-fluoroproline residues on PPI/PPII interconversion kinetics

Since PPII helices are predominated in aqueous solution and PPI helices are favored in *n*-propanol, for the estimation of polyproline interconversion rates, we measured PPI → PPII transition in the solution of 90 % (v/v) phosphate buffer and 10 % *n*-propanol, and PPII → PPI transition in the solution of 95 % (v/v) *n*-propanol and 5 % phosphate buffer. Similar to the method described in our previous study (Chiang et al. 2009), the peptides were first pre-incubated in aqueous solution or *n*-propanol to secure the formation of PPII or PPI helices, and were then transferred to the solvent favoring another conformation. Time-dependent CD spectra were taken upon switching solvent to monitor the signal changes and determine the conversion rate constants.

Upon dissolving pre-incubated PPI helices into aqueous solution, we monitored the conversion of PPI to PPII by taking a series of far-UV CD spectra at different time. As shown in Fig. S3 (in the supplementary material), all the peptides exhibit a transition from PPI conformation (with a strong positive band around 213 nm and a weak negative band around 230 nm) to PPII conformation (with a positive band between 220 and 230 nm and an intense negative band between 200 and 210 nm) with an isosbestic point approximately at 222 nm. The results are consistent with our previous observation that only two conformations, i.e., PPI and PPII helices, exist in solution during the structural transformation. Plotting the positive maximum of PPI signal at 213 or 214 nm versus time showed an exponential decay for each peptide. As shown in Fig. 2, the PPI → PPII conversion rate constant for each peptide was determined by fitting the curve into a first-order exponential decay (Eq. 1),

$$\theta(t) = \theta_o \exp(-kt) + b \quad (1)$$

where $\theta(t)$ is the molar ellipticity at time t, θ_o is the molar ellipticity at time zero, k is the conversion rate constant and b is a baseline correction term. As shown in Table 2, the rate constants of PPI → PPII conversion for all the peptides are in the order of 10^{-5} s^{-1} except for the P11 peptide (rate constant k is 8.83 × 10^{-6} s^{-1} for P11). The results indicate that all the Flp and flp-substituted polyproline peptides have a faster PPI → PPII transition rate than P11 and substitution sites appear not to play a critical role affecting this process. We further calculated the transition energy changes ($\Delta\Delta G^{\neq}$) between P11 and the fluoroproline containing peptides by Eq. 2,

$$\Delta\Delta G^{\neq} = -RT \ln(k_{mut}/k_{P11}) \quad (2)$$

where k_{mut} is the rate constant for the polyproline peptide containing Flp or flp, k_{P11} is the rate constant for P11, R is gas constant, and T is absolute temperature (277 K). The calculated $\Delta\Delta G^{\neq}$ values for PPI → PPII conversion are listed in Table 2. The data reveal that all the Flp or flp containing peptides decrease the transition barrier of PPI → PPII conversion by 0.70–0.99 kJ mol^{-1} and there is no distinction between the substitutions with Flp and flp. The results are consistent with our previous finding that stereoelectronic effects might not be critical in mediating

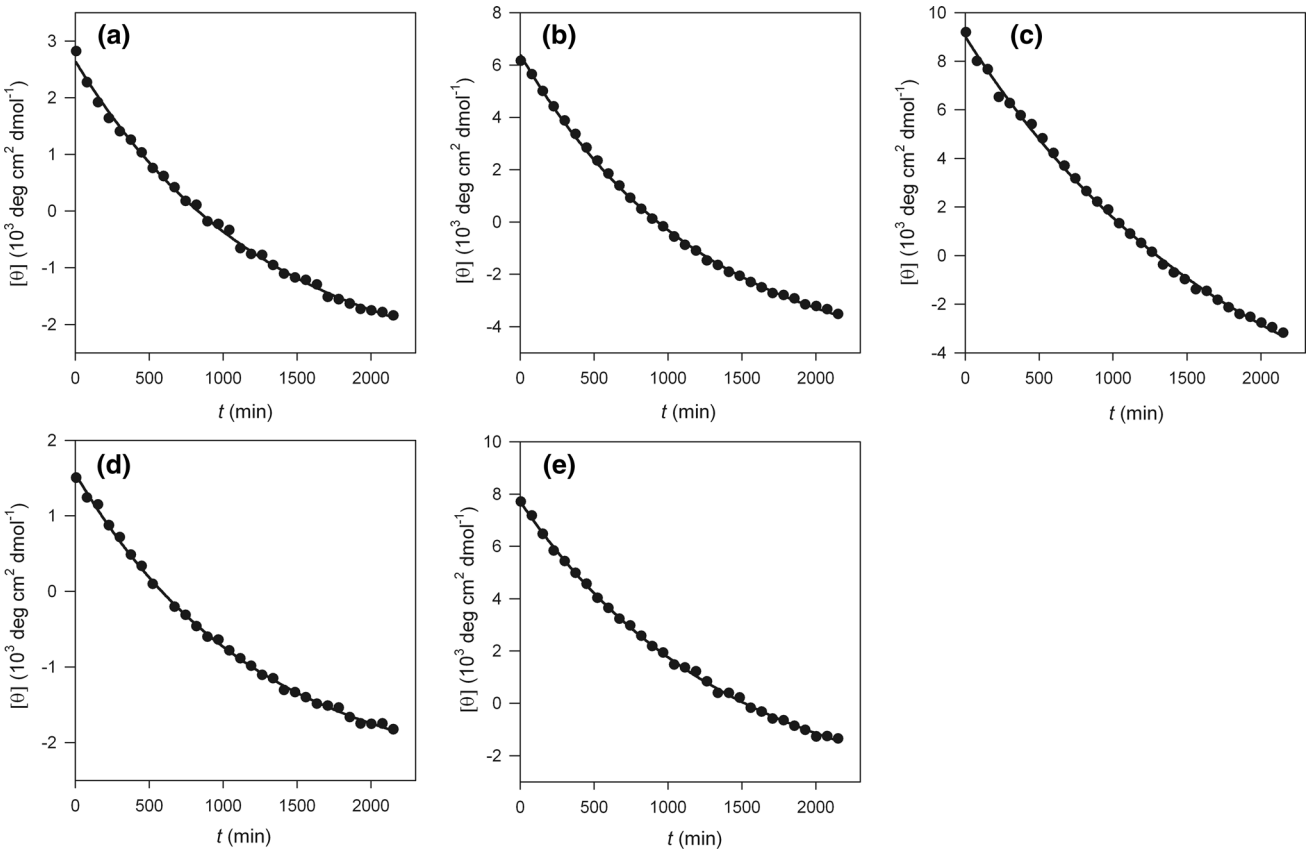

Fig. 2 A plot of molar ellipticity at 213 or 214 nm versus time for **a** N-Flp-P11, **b** C-Flp-P11, **c** P11, **d** N-flp-P11, and **e** C-flp-P11 in 90 % (v/v) aqueous solution at 4 °C

Table 2 Rate constants of PPI ↔ PPII interconversion and the transition energy changes for polyproline peptides at 4 °C

Peptide	PPI → PPII rate constant (s^{-1})[a]	$\Delta\Delta G^{\neq}_{PPI \rightarrow PPII}$ (kJ mol^{-1})[b]	PPII → PPI rate constant (s^{-1})[a]	$\Delta\Delta G^{\neq}_{PPII \rightarrow PPI}$ (kJ mol^{-1})[b]
N-Flp-P11	12.96 (0.60) × 10^{-6}	−0.88	9.18 (1.43) × 10^{-6}	0.10
C-Flp-P11	13.55 (0.32) × 10^{-6}	−0.99	4.93 (0.34) × 10^{-6}	1.53
P11	8.83 (0.47) × 10^{-6}	–	9.59 (0.32) × 10^{-6}	–
N-flp-P11	13.58 (0.42) × 10^{-6}	−0.99	9.72 (0.87) × 10^{-6}	−0.03
C-flp-P11	11.96 (0.34) × 10^{-6}	−0.70	71.08 (12.25) × 10^{-6}	−4.61

[a] The values in parentheses are the standard errors to the fit

[b] $\Delta\Delta G^{\neq}$ values were calculated by Eq. 2, $\Delta\Delta G^{\neq} = -RT\ln(k_{mut}/k_{P11})$, where k_{mut} is the rate constant for the polyproline peptides with Flp or flp substitutions and k_{P11} is the rate constant for P11

the transition barrier of PPI → PPII conversion. Moreover, no discriminative effects on PPI → PPII transition between N-Flp-P11 and C-Flp-P11 or between N-flp-P11 and C-flp-P11 were observed, further suggesting that terminal stereoelectronic effects are not a key factor affecting the PPI → PPII conversion kinetics.

Likewise, we transferred the pre-incubated peptides from aqueous solution (PPII helices) to 95 % (v/v) *n*-propanol and measured a series of time-dependent CD spectra. As shown in Fig. S4 (in the supplementary material), the far-UV CD spectra showed that all the peptides gradually changed from PPII helices to PPI helices. A plot of the molar ellipticity at 227 or 228 nm (the characteristic band of PPII conformation) versus time shows an exponential decay for each peptide (Fig. 3). Fitting the curve into a first-order exponential decay (Eq. 1) can obtain the rate constant of PPII → PPI conversion. Changes in the transition energy barrier of PPII → PPI conversion can also be calculated using determined rate constants and Eq. 2. The parameters derived from the time-dependent CD

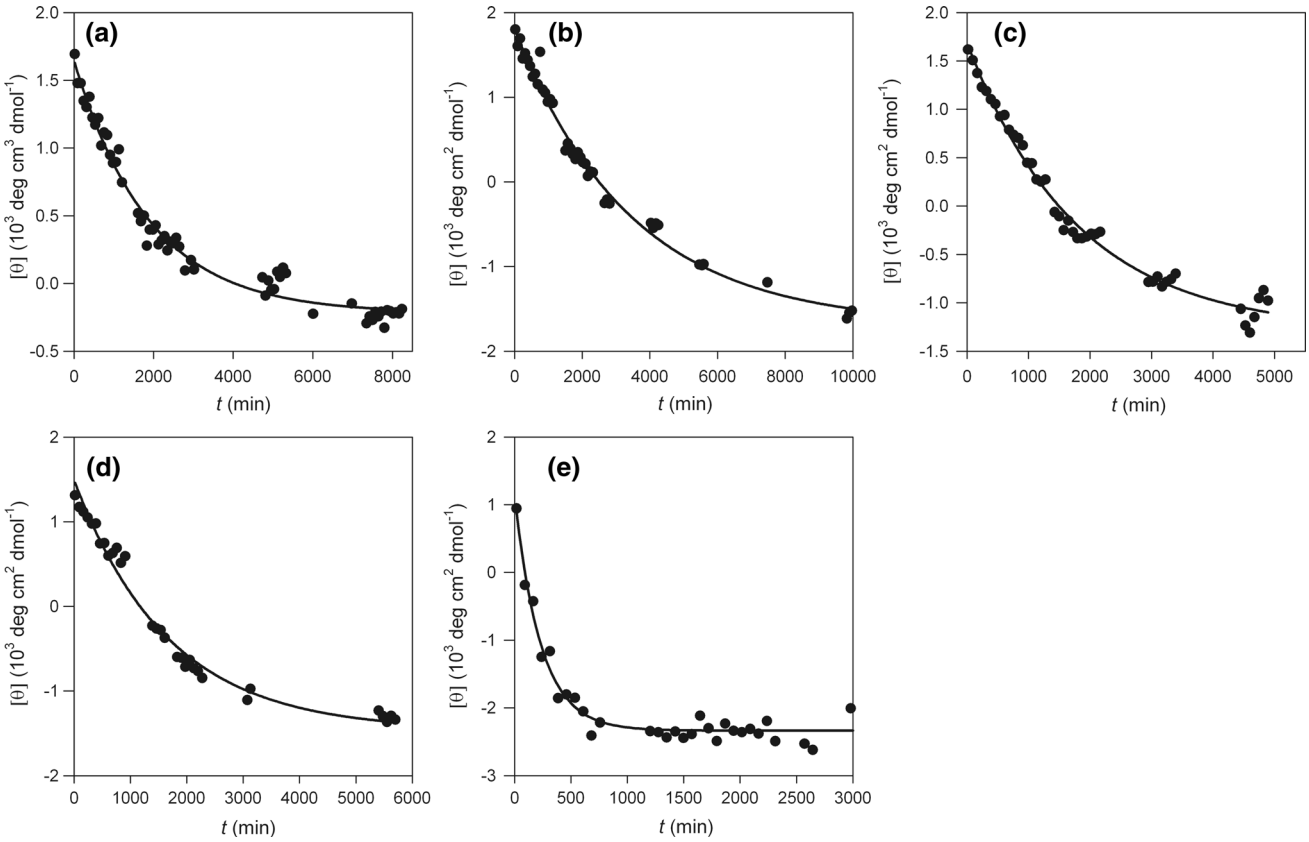

Fig. 3 A plot of molar ellipticity at 227 or 228 nm versus time for **a** N-Flp-P11, **b** C-Flp-P11, **c** P11, **d** N-flp-P11, and **e** C-flp-P11 in 95 % (v/v) *n*-propanol at 4 °C

measurements are summarized in Table 2. As shown in Fig. 3 and Table 2, N-Flp-P11 and C-Flp-P11 have a slower PPII → PPI conversion rate than does P11, while N-flp-P11 and C-flp-P11 have a greater PPII → PPI conversion rate than P11. It is evident that stereoelectronic effects induce distinct consequences on the PPII → PPI conversion rate, well concurring with our previous observations that an electron-withdrawing group on the 4*R* position (Flp) has a high propensity to form C^{γ}-*exo* pucker and *trans* peptide bonds and increases the PPII → PPI transition barrier while an electron-withdrawing group in the 4*S* position (flp) disfavors C^{γ}-*exo* pucker and reduces the PPII → PPI transition barrier (Chiang et al. 2009).

However, compared to P11, the $\Delta\Delta G^{\neq}$ for N-Flp-P11 and N-flp-P11 is only 0.10 and −0.03 kJ mol^{-1} respectively, indicating that the incorporation of Flp and flp into the N terminus just slightly affect the PPII → PPI conversion kinetics and stereoelectronic effects do not have a significant impact on the transition barrier from the N-terminal side of the peptide. In contrast, the rate constants of PPII → PPI conversion for C-Flp-P11 and C-flp-P11 are dramatically different from that of P11. The $\Delta\Delta G^{\neq}$ is 1.53 kJ mol^{-1} for C-Flp-P11 and -4.61 kJ mol^{-1} for C-flp-P11, showing that Flp and flp would have a greater effect on the PPII → PPI transition barrier when they are located at the C terminus of a polyproline peptide. The data show that the stereoelectronic effects in modulating the PPII → PPI transition barrier are directional, i.e., fluoroproline residues can impose stronger stereoelectronic effects from the C terminus than from the N terminus to affect the PPII → PPI conversion kinetics. This result is consistent with the *trans–cis* isomerization mechanism of a polyproline peptide which initiates from the C terminus (Lin and Brandts 1980). We can interpret this result by the illustration in Fig. 4, where the C-terminal flp residue favoring a *cis* peptide bond can serve as the driving force to facilitate the formation of a PPI helix when the helix starts folding from the C terminus. As to Flp, its great propensity in forming a *trans* peptide bond makes it disfavored in PPI conformation and thereby impedes the transformation of PPII to PPI when a PPI helix initiates its fold at the C terminus. In addition, the impact of N-terminal Flp and flp substitutions on the *trans–cis* isomerization rate is relatively small, further supporting the C to N mechanism of PPII → PPI transformation.

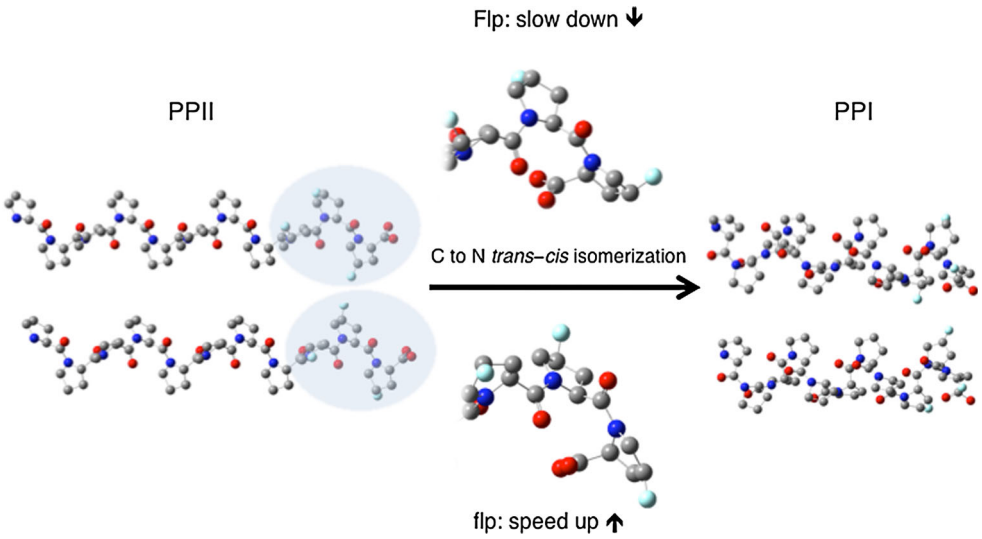

Fig. 4 Illustration of C-terminal stereoelectronic effects on the transformation of PPII → PPI. Flp represents (2*S*,4*R*)-4-fluoroproline, flp represents (2*S*,4*S*)-4-fluoroproline, and fluorine atoms are shown in cyan. The molecular models were generated by the software of *Gaussian 09*

We can further correlate the stability of PPII conformation with the PPII → PPI conversion rate by assuming the transition states of PPII → PPI conversion are the same in energy and then comparing the relative energy of the PPII ground states for P11, C-Flp-P11, and C-flp-P11. From such a comparison, we can find that the PPII stability of C-Flp-P11 is greater than that of P11 and C-flp-P11 by 1.53 and 6.14 kJ mol^{-1}, respectively, leading to a relatively slow PPII → PPI conversion process for C-Flp-P11. Therefore, upon the incorporation of 4-fluoroprolines, the C-terminal effects observed on the conversion kinetics of PPII → PPI are actually related to the stability of PPII conformation. The larger impact of stereoelectronic effects from the C-terminal end on the conversion kinetics of PPII → PPI transformation is also consistent with that the C-terminal stereoelectronic effects play a more critical role in modulating PPII stability. Combining the thermodynamic and kinetic data, it suggests that the mediation of a PPII helix by noncovalent interactions, such as stereoelectronic effects, can be more effective from its C-terminal side.

Conclusions

In this work, we have used (4*R*)-fluoroproline and (4*S*)-fluoroproline containing polyproline peptides to demonstrate that polyproline conformation is significantly affected by the C-terminal stereoelectronic effects, including PPII stability and the kinetics of PPII → PPI conversion. In contrast with the C-terminal 4-fluoroproline residues, the N-terminal fluorinated proline derivatives do not exhibit a similar impact on polyproline conformation, suggesting stereoelectronic effects cannot predominately regulate polyproline conformation from the N-terminal side. The kinetic data are consistent with the C to N mechanism of *trans–cis* isomerization for a polyproline peptide, and our kinetic study may also provide another approach to unravel the folding mechanism of a PPI helix. Furthermore, unlike the consequences of terminal stereoelectronic effects on PPII → PPI transformation, such influences are not observed on the kinetics of PPI → PPII conversion, supporting our previous argument that stereoelectronic effects are not a key factor in determining the rate of PPI → PPII transformation (Chiang et al. 2009). Although polyproline conformation has been widely studied in the past decades, our current work reports the first study of terminal stereoelectronic effects on polyproline structure. Our finding reveals a new insight into the subtle role of stereoelectronic effects on the stability and interconversion kinetics of polyproline structures, and should be of great use to rationally design PPI or PPII helices for specific applications.

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Conflict of interest The authors declare that they have no conflict of interest.

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