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Temperature-induced transition between polyproline I and II helices: quantitative fitting of hysteresis effects‡

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The conformational properties of the polyproline I (PPI) helix of oligoprolines toward heating were examined. Oligoproline H-Pro$_{12}$-NH$_2$ served as a model which adopts in n-PrOH a pronounced PPI conformation with all *cis* amide bonds, whereas a polyproline II (PPII) conformation with all *trans* amide bonds is predominant in pure aqueous buffer. CD spectroscopic studies revealed that a conformational change from the PPI to the PPII helix takes place upon heating and back to the PPI helix upon cooling. This conformational transition cycle is characterized by a strong hysteresis. With a quantitative fitting of the experimentally observed hysteresis loops by a newly developed iterative integration with different starting conditions, kinetic and thermodynamic parameters for the transition from the PPI to the PPII helical conformation were determined. The transition is as expected for *cis*–*trans* isomerizations of amide bonds comparatively slow ($k = 0.003\ s^{-1}$ at 80 °C) and characterized by an activation energy E_a of 81.1 ± 3.6 kJ mol^{-1}. Thermodynamically, the transition from the PPI to the PPII helix is characterized by a positive standard enthalpy ($\Delta H^0 = 33.5 \pm 2.1$ kJ min^{-1}) and a positive standard entropy ($\Delta S^0 = 102 \pm 6.6$ J mol^{-1} K^{-1}).
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Keywords: PPI and PPII helices; CD spectroscopy; hysteresis; oligoproline; peptides

Introduction

Oligoprolines are unique among peptides consisting of natural amino acids as they switch between two distinctly different helical conformations depending on their environment [1–13]. In aqueous solution, oligoprolines adopt the polyproline II (PPII) helix, whereas in more hydrophobic solvents such as aliphatic alcohols, the polyproline I (PPI) helix is favored (Figure 1) [1–13]. The transition of these two polyproline helices into each other goes along with an isomerization of all amide bonds of the peptide backbone – in the PPI helix, the amide bonds are in *cis*, whereas in the PPII helix they are in *trans* conformations. As a consequence, oligoprolines are valuable model compounds (i) to study *cis*–*trans* isomerizations of peptide bonds which play important roles in, for example, signaling and protein folding [14–21] and (ii) for understanding the conformational properties of the PPII conformation which is widespread in natural proteins and is the basic structure of the single strands of collagen [22–28]. Furthermore, they are attractive as conformationally well-defined and switchable molecular scaffolds provided that suitable sites for functionalization are installed [10–12].

The PPI helix is right-handed with a helical pitch of 5.6 Å per turn and backbone dihedral angles ϕ and ψ of -75° and $+160^\circ$, respectively [1–3, 29]. The more extended left-handed PPII helix has a helical pitch of 9.4 Å and dihedral angles ϕ and ψ of -75° and $+145^\circ$, respectively [1–3, 30]. The two helical conformations can be easily distinguished by CD spectroscopy [1–13, 31]. Due to the importance of the PPII helix in many biological processes, a lot of research has focused on the PPII helix. Comparatively little is known about the properties of the PPI helix [1–13].

Previously, we have shown that the PPI helix is stabilized relative to the PPII helix by positively charged functional groups at the *N*-terminus and negatively charged functional groups at the *C*-terminus [13]. In the present work, we studied the conformational properties of the PPI helix upon changing the temperature. We demonstrate that a transition from the PPI to the PPII helix takes place upon heating and back to the PPI helix upon cooling. This conformational transition is characterized by a strong hysteresis. Quantitative fitting of the experimentally observed hysteresis loops by a kinetic mechanism allowed to determine the van't Hoff enthalpy of the temperature-induced transition between the PPI and PPII helices as well as rate constants and the activation energy.

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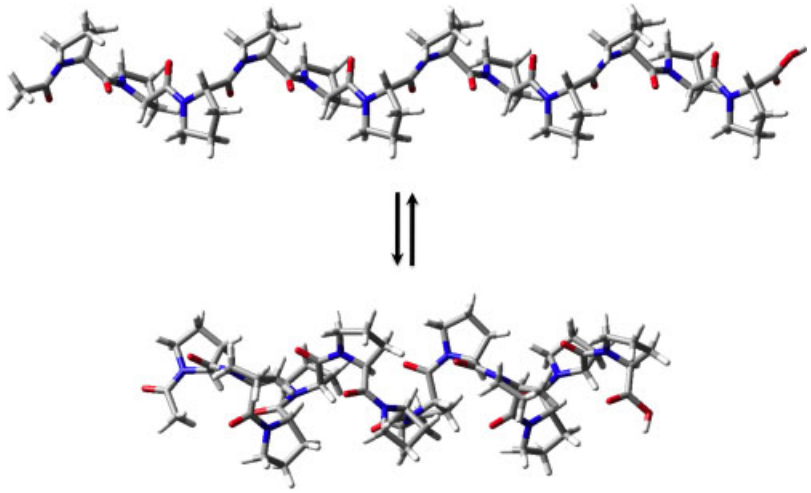

Figure 1. Equilibrium between the PPII helix (top) and the PPI helix (bottom).

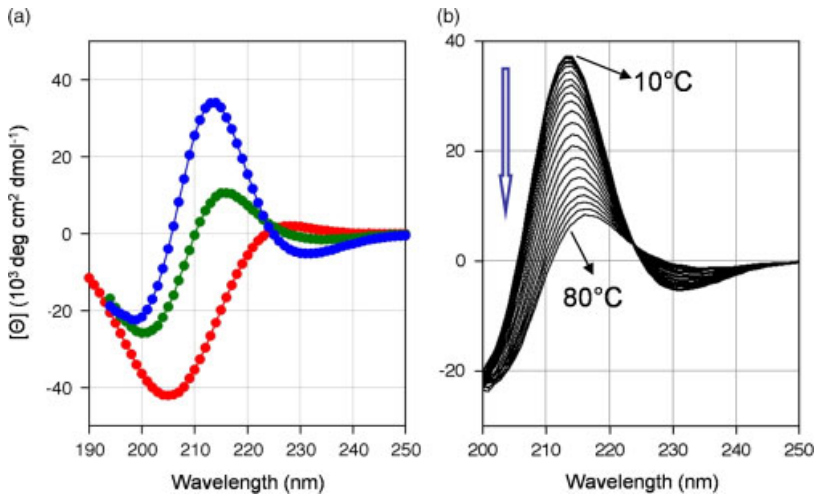

Figure 2. CD spectra of H-Pro_{12}-NH_2 (50 μM): (a) at 25 °C in aqueous phosphate buffer (10 mM, pH 7.2) (red) and mixtures of 95% n-PrOH (green) and 99% n-PrOH (blue) in phosphate buffer, respectively; (b) in 99% n-PrOH and 1% phosphate buffer recorded in 2.5 °C intervals from 10 to 80 °C.

Results and Discussion

Temperature-Induced Switching from the PPI to the PPII Helix

Solvent-induced isomerizations of the PPI and PPII helices into each other are well known [1–13]. For example, the oligoproline H-Pro_{12}-NH_2 switches between the PPII and the PPI helix when dissolved in different mixtures of aqueous phosphate buffer and n-PrOH (Figure 2(a)). In aqueous buffer, the typical spectrum of a PPII helix with a minimum at 205 nm and a weak positive band at 228 nm is observed (Figure 2(a), red). In 99% n-PrOH in aqueous buffer, the spectrum is typical for a PPI helix with a strong maximum at 213 nm and a weak negative band around 232 nm (Figure 2(a), blue). In mixtures of these two solvents, both conformations occur (Figure 2(a), green). Upon heating the peptide dissolved in aqueous buffer, hardly any change in the CD spectrum takes place demonstrating the thermal conformational stability of the PPII helix in aqueous buffer [13]. In contrast, significant changes in the CD spectra were observed upon heating the solution of oligoproline H-Pro_{12}-NH_2 in 99% n-PrOH, an environment in which the PPI conformation is favored over the PPII conformation (Figure 2(b)). This demonstrates that the PPI helix is not thermostable under these conditions. A comparison with the CD data in different solvent compositions (Figure 2(a)) illustrates that a transformation of the PPI into the PPII helix takes place upon increasing the temperature. This transition is not complete within the temperature range accessible with n-PrOH (boiling point at 97 °C), but a significant fraction of the PPII helix is formed. The isodichroic point at 224 nm indicates that only a two-state equilibrium is involved in this shift from the PPI to the PPII conformation.

When the sample was cooled down again, the conformational switch back to the PPI helix took place. Interestingly, at the end point of the cooling process, the observed CD spectrum differs significantly from that of the original starting point. A strong hysteresis is observed in this heating and cooling cycle. Figure 3 illustrates how the CD signal at 213 nm (characteristic maximum of the CD spectrum of the PPI helix) changes when the temperature is first increased from 10 to 90 °C and then decreased back to 10 °C at three different heating and cooling rates of 0.1, 0.5 and 1 °C min^{-1}, respectively. The data demonstrate that the hysteresis depends significantly on the heating and cooling rates.

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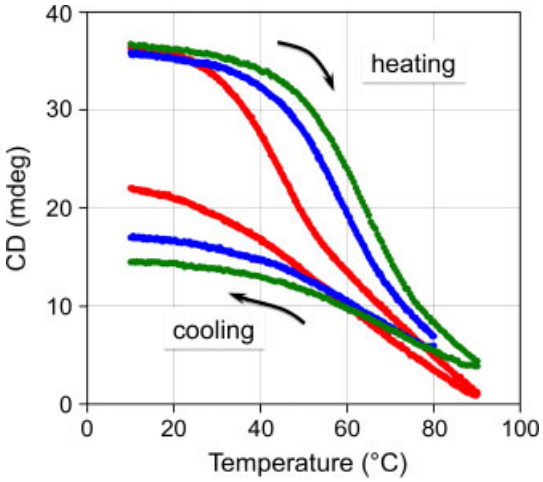

Figure 3. Change of the CD signal at 213 nm of H-Pro$_{12}$-NH$_2$ (37 μM in a solution of 99% n-PrOH and 1% phosphate buffer, pH = 7.2) upon heating and cooling with rates of 1 °C min^{-1} (green), 0.5 °C min^{-1} (blue) and 0.1 °C min^{-1} (red).

The faster the temperature is changed, the more pronounced is the observed hysteresis. (Since the transition to the PPII helix is not complete, no plateau is reached at the highest accessible temperature.)

The observed hysteresis suggests that the *cis–trans* isomerization of the amide bonds involved in the transition from the PPI to the PPII helix and vice versa is slow compared to the change in the temperature. Hysteresis effects are also known for other systems of biological relevance, for example the folding/unfolding of the collagen triple helix [32–34] and α-helical coiled-coil structures of SNARE proteins [35]. We therefore sought to obtain a deeper understanding of the transition between the PPI and the PPII helix and fitted the experimental data with a newly developed kinetic model for the quantitative description of hysteresis effects ([32]; within this publication, the newly developed formalism for the quantitative description of hysteresis effects of even complex systems is applied to collagen) to determine thermodynamic and kinetic parameters that characterize this molecular switch.

Analysis of the Hysteresis – Determination of Thermodynamic and Kinetic Parameters

A first-order kinetic model that assumes an all-or-none transition between two states with first-order rate constants k_1 and k_{-1} proved to describe the experimental observations very well. The following equations were derived to describe the equilibrium between the PPI and PPII helices.

$$\frac{\mathrm{d[PPII]}}{\mathrm{d}t} = k_1 \cdot [\mathrm{PPI}] - k_{-1} \cdot [\mathrm{PPII}] \quad (1)$$

With the fraction in PPI conformation $F = [\mathrm{PPI}]/c_0$ and $c_0 = [\mathrm{PPI}] + [\mathrm{PPII}]$, the total concentration of the peptide is as follows:

$$\frac{\mathrm{d}F}{\mathrm{d}t} = -k_1 \cdot F + k_{-1} \cdot (1 - F) \quad (2)$$

The mean molar ellipticity θ (T) was described in F as follows:

$$\theta(T) = \theta_{\mathrm{PPII}} + F \cdot (\theta_{\mathrm{PPI}} - \theta_{\mathrm{PPII}}) \quad (3)$$

The temperature was calculated from the starting temperature T_{start}, the heating rate ρ and the time t:

$$T = T_{\mathrm{start}} + \rho \cdot t \quad (4)$$

The activation energy (E_a) for the transition PPI → PPII is derived from the temperature dependence of the rate constants by the Arrhenius equation, k_{10} was introduced as a reference rate constant at 0 °C (T_0 = 273 K). R is the gas constant:

$$k_1(T) = k_{10} \cdot e^{\left[\frac{E_a}{R} \cdot \left(\frac{1}{T_0} - \frac{1}{T}\right)\right]} \quad (5)$$

Furthermore, the equilibrium constant K was calculated from the standard values of enthalpy ΔH^0 and entropy ΔS^0:

$$K(T) = \mathrm{e}^{\left(\frac{-\Delta H^0 - T \cdot \Delta S^0}{RT}\right)} \quad (6)$$

with

$$k_{-1} = k_1 \cdot K \quad (7)$$

Heating and cooling were described by the same mechanism, only with different starting conditions, $F = 1$ at $t = 0$ for heating and $F \approx 0.2$ at $t = 0$ for cooling. A value of $F = 0.2$ was estimated as the transition from the PPI to the PPII helix was not complete at the maximum temperature and proved to fit best for the analysis of the experimental data.

The resulting differential equation was iteratively solved by the MicroMath Scientist for Windows Version 2.01 program (MicroMath, Inc. Saint Louis, MO, USA). The evaluation scheme is given in the Supporting Information. The following parameters were fitted by independent variation to the experimental curves: the standard enthalpy ΔH^0, standard entropy ΔS^0, the rate constant k_{10} for the transformation of the PPI helix to the PPII helix at 0 °C and the corresponding activation energy E_a (Table 1). Figure 4 shows the very good match of the fitting with the experimental data. The values for the fitted parameters do not depend significantly on the heating/cooling rate (0.1, 0.5 or 1 °C min^{-1}) or the concentration (111 or 37 μM) (Table 1).

The transition from the PPI to the PPII helix was found to be a comparatively slow process with kinetic parameters of $k_1 = 8 \times 10^{-7}$ s^{-1} at 0 °C and $k_1 = 0.003$ s^{-1} at 80 °C (derived from Eqn (5) with k_{10} and E_a). To validate these rate constants determined by the hysteresis model, an additional temperature jump experiment was performed. Within this experiment, the CD spectrum of a sample equilibrated in 99% n-PrOH at 10 °C and quickly heated to 80 °C was monitored and the kinetic data were fitted to a first-order reaction mechanism. The obtained rate constant of $k_1 = 0.005$ s^{-1} (at 80 °C) is in good agreement with that derived from the hysteresis experiment ($k_1 = 0.003$ s^{-1} at 80 °C) demonstrating the validity of the model.

An activation energy of 81 kJ mol^{-1} was determined which is typical for *cis–trans* isomerizations of Xaa–Pro bonds and previously examined transitions of oligoprolines [14–16, 36, 37]. This high activation energy is very important for the occurrence of the hysteresis as it gives rise to the slow conversion at low temperatures. At very low temperatures, a thermodynamically unstable form of the oligoproline may even be kept frozen for long time because of the strong hysteresis. For example, an experiment was performed (data not shown) in which H-Pro$_6$-OH in the PPII conformation was dissolved in a mixture of 95%

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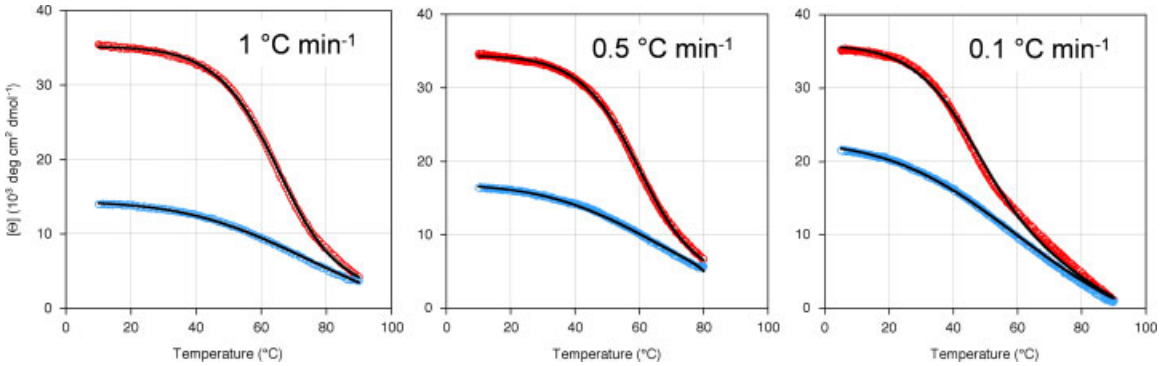

Figure 4. Experimental CD signal of H-Pro$_{12}$-NH$_2$ at 213 nm for heating (red) and cooling (blue), as well as the calculated curve (–) at a concentration of 37 μM with three different heating/cooling rates (left: 1 °C min^{-1}, middle: 0.5 °C min^{-1}, right: 0.1 °C min^{-1}).

Table 1. Parameters determined from the fitting of the kinetic hysteresis

Concentration Heating/cooling rate	37 μM 1 °C min^{-1}	37 μM 0.5 °C min^{-1}	37 μM 0.1 °C min^{-1}	111 μM 0.5 °C min^{-1}	Average values[a]
ΔH^0/kJ mol^{-1}	30.6	35.6	32.2	35.4	33.5 ± 2.1
ΔS^0/J mol^{-1} K^{-1}	94.0	109	98.1	109	102 ± 6.6
k_1/s^{-1} (T = 273 K)	1.0 × 10^{-6}	7.5 × 10^{-7}	7.8 × 10^{-7}	7.0 × 10^{-7}	(8 ± 1) × 10^{-7}
E_a/kJ mol^{-1}	82.2	84.3	74.9	82.8	81.1 ± 3.6

[a] Average values with standard deviation derived from four experiments.

n-PrOH in phosphate buffer at 0 °C. Under these conditions, the conformation should convert to the PPI helix but it remained in the PPII conformation for days at the low temperature. Only when the rate of isomerization was increased by raising the temperature, conversion to the PPI helix was observed. A further temperature increase then led to stabilization of the PPII helix. As a result, a hill-shaped transition curve due to the transition PPII–PPI–PPII was observed by monitoring the CD signal at 213 nm. This complex transition curve is also well described by Eqns (1)–(7) with appropriate starting conditions of integration. The experiment demonstrates that complex switching events may be caused by the hysteresis.

Interestingly, the transition from the PPI to the PPII helix is endothermic with a standard reaction enthalpy of ΔH^0 = 33.5 kJ mol^{-1} (Table 1). This is in agreement with ab initio calculations that predict an energetic favorisation of the PPI over the PPII helix in the gas phase [13]. The increase in enthalpy is accompanied by an increase in entropy of ΔS^0 = 102 J mol^{-1} K^{-1}. As a result, the system reacts with an increasing population of the PPII conformer toward heating.

The data demonstrate that the PPI to PPII transition is an example of a kinetic hysteresis. The external temperature change imposed on the system is faster than the slow *cis–trans* isomerization. Thus, the system responds with a delay. This kinetic nature of the hysteresis is supported by the fact that the same kinetic mechanism describes the forward and backward branches of the hysteresis loop with the only difference of using different starting conditions for the integration.

Conclusions

In summary, we have shown that upon heating oligoprolines in a PPI helical conformation, a conversion to the PPII conformation takes place. The transition back to the PPI helix upon cooling is characterized by a strong hysteresis which is due to the high activation energy (E_a ~ 81 kJ mol^{-1}) for the *cis–trans* isomerization of the amide bonds. The experimentally observed hysteresis is well fitted by the differential equation derived for a two-state equilibrium changing only the starting conditions of the integration for the heating and cooling processes. The transition PPI → PPII goes along with a positive standard enthalpy of ~33.5 kJ mol^{-1} and an increasing standard entropy of ~102 J mol^{-1} K^{-1}. This insight into the temperature-induced conformational switch between the PPI and the PPII helices is useful for the application of functionalizable azidoproline-containing oligoprolines as switchable synthetic scaffolds [10–13]. Based on our data, it is tempting to speculate that all transitions involving *cis–trans* isomerizations as rate-limiting steps are characterized by a kinetic hysteresis. Thus, the data are also relevant for a deeper understanding of *cis–trans* isomerizations of Xaa–Pro bonds in protein folding and the role of PPII segments in unfolded polypeptide chains [14–16, 22–24].

Experimental Procedures

Synthesis of the Peptide H-Pro$_{12}$-NH$_2$: The peptide H-Pro$_{12}$-NH$_2$ was synthesized on Rink Amide resin on a 100 μmol scale by standard solid-phase synthesis using a Syro I Peptide Synthesizer (MultiSynTech GmbH, Witten, Germany). For the couplings Fmoc-Pro-OH (4 eq), HCTU (4 eq) dissolved in DMF and ^{i}Pr$_2$NEt (16 eq) were added to the amino-functionalized resin (≈100 mM). The mixture was agitated for 1 h and washed with DMF (3×). For Fmoc-deprotections: A solution of 20% piperidine in DMF was added to the resin and the reaction mixture was agitated for 3 min, drained and the piperidine treatment repeated for 10 min. Finally the resin was washed with DMF (5×). The solid supported peptide was

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cleaved off the resin by stirring in a mixture of trifluoroacetic acid and CH_2Cl_2 for 1 h and a second time for 20 min. All volatiles of the combined filtrates were removed under reduced pressure. The peptide was precipitated with Et_2O and purified by preparative HPLC on a LiChrospher RP-18e 5 μM (250 mm × 10 mm) column from Merck.

Analytical data: HPLC: t_R = 12.7 min; linear gradient: 7% to 31% CH_3CN in 0.1% TFA/H_2O over 20 min at a flow rate of 1 ml/min. HRMS (ESI): m/z = 1182.6677 calcd for $C_{60}H_{88}N_{13}O_{12}$ $[M+H]^+$; found: 1182.6652 (Bruker FTMS 4.7T BioAPEX II instrument).

CD Spectroscopy: A Chirascan Circular Dichroism Spectrometer (Applied Biophysics Ltd, Leatherhead, UK) equipped with a peltier temperature control device (Alpha Omega Instruments, Cumberland, USA) was used for CD measurements. CD spectra were recorded using a spectral bandwidth of 1 nm with a time constant of 5 s and a step resolution of 1 nm. CD data are reported as mean residual molar ellipticities (Θ_{MRW} in deg cm^2 $dmol^{-1}$). A Quartz cell with a path length of 2 mm was used. All samples were equilibrated for at least 12 h before measurement. For hysteresis experiments equilibrated samples were heated with a constant heating rate (1, 0.5 or 0.1 °C min^{-1}). The CD signal at 213 nm was recorded every 0.2 °C (0.5 °C with a heating rate of 1 °C min^{-1}) starting from 10 °C with a time constant of 3 s up to 80 or 90 °C. Afterward the temperature was decreased with the same rate. For the temperature jump experiments the cell was equilibrated at 10 °C and then introduced in the CD instrument that was hold at 80 °C. The CD signal was followed over 3000 s recording a value every 3 seconds.

Supporting information

Supporting information may be found in the online version of this article.

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