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Force-Induced Prolyl Cis−Trans Isomerization in Elastin-like Polypeptides

Alexei Valiaev,[†,#] Dong Woo Lim,[‡,#] Terrence G. Oas,[§] Ashutosh Chilkoti,[‡,#] and Stefan Zauscher*[,†,#]

Contribution from the Department of Mechanical Engineering and Materials Science, Department of Biomedical Engineering, Department of Biochemistry, and Center for Biologically Inspired Materials and Materials Systems, Duke University, Durham, North Carolina 27708

Received January 8, 2007; E-mail: zauscher@duke.edu

Abstract: Elastin-like polypeptides (ELPs) are stimulus-responsive polymers that contain repeats of five amino acids, Val-Pro-Gly-Xaa-Gly (VPGXG), where Xaa is a guest residue that can be any amino acid with the exception of proline. While studying the conformational mechanics of ELPs over a range of solvent conditions by single-molecule force spectroscopy, we noticed that some force−extension curves showed temperature-independent, extensional transitions that could not be fitted with a freely jointed chain or worm-like chain model. Here we show that the observed molecular elongation results from the force-induced peptidyl−prolyl cis−trans isomerization in prolines, which are repeated every fifth residue in the main chain of ELPs. Control experiments with poly(L-proline) demonstrate the similarity of the conformational transition between poly(L-proline) and ELPs. In contrast, the force−extension behavior of poly(L-lysine) showed no deviation in the relevant force range. Force−extension curves in hysteresis experiments showed an elongational difference between extension and relaxation pathways that suggests that the cis conformational state of the prolines could be exhausted on the time scale of the experiment. We present further computational evidence for this mechanism by Monte Carlo simulation of the force−extension behavior using an elastically coupled, two-state model. We believe ours is the first demonstration of force-induced prolyl cis−trans isomerization in proline-containing polypeptides. Our results suggest that single-molecule force spectroscopy could provide an alternate means to assay this important conformational transition in polypeptides.

Introduction

Research of the peptidyl−prolyl cis−trans isomerization is important for understanding the biologically active conformation of proteins, protein stability, and folding pathways.[1] Protein folding and unfolding often involves a rate-limiting cis−trans isomerization of prolines,[2,3] and in some proteins proper folding occurs only when all prolines are in the trans state.[3,4] Because of the high activation energy barrier (∼60−80 kJ/mol),[2] prolyl cis−trans isomerization occurs slowly at equilibrium. In the absence of structural constraints, both the cis and trans isomers are significantly populated, since the difference in the Gibbs free energy between the two states is small (<8 kJ/mol).[2,5]

In most of the previously reported experiments, cis−trans isomerization was catalyzed in a chymotrypsin-coupled, proline isomerase assay;[5,6] however, certain proteins cannot be catalyzed using the known peptidyl−prolyl cis−trans isomerase (PPIase). For example, it was shown that folding of intact RNase A and thirodoxin could not be induced by using PPIase, although both of these proteins involve cis−trans isomerization during folding.[2] This lack of catalysis was explained by the random formation of ordered structures that close the interaction sites for the prolyl isomerase.[2] For proteins that lack PPIase-induced isomerization, force, as shown here, could potentially serve as an alternative trigger for studying prolyl cis−trans isomerization. Prolyl cis−trans isomerization induced by mechanical force can also be significant in biology. For example, in a recent paper, it was proposed that cis−trans peptide bond isomerization is intimately involved in the chemical-to-mechanical energy transduction in the motor protein myosin.[7] It is difficult to systematically study force-induced prolyl cis−trans isomerization on proteins with highly diverse sequences and folding behaviors, and access to a simpler, experimentally more tractable system is thus desirable.

Motivated by this rationale, we chose recombinant elastin-like polypeptides (ELPs) as the model system to study the peptidyl−prolyl cis−trans isomerization by single-molecule force spectroscopy (SMFS). ELPs are stimulus-responsive polymers that contain repeats of five amino acids, Val-Pro-Gly-

[†] Department of Mechanical Engineering and Materials Science.
[‡] Department of Biomedical Engineering.
[§] Department of Biochemistry.
[#] Center for Biologically Inspired Materials and Materials Systems.

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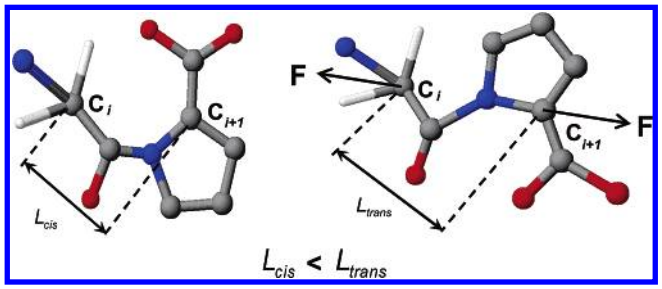
Scheme 1. Cis and Trans Conformational States of Proline[a]



[a] The force-induced prolyl cis−trans isomerization increases the $C_i^{\alpha}/C_{i+1}^{\alpha}$ atom distance (an elongation of 2 Å per proline was assumed throughout this work).

Xaa-Gly (VPGXG), where Xaa is a guest residue that can be any amino acid with the exception of proline. ELPs provide an ideal model system because they contain a high concentration (∼12%) of prolines that are precisely spaced along the polypeptide chain. The high concentration and invariant location of prolines in ELPs make it an ideal candidate to study the effect of cis−trans isomerization on the force−extension of proline-containing systems. Furthermore, ELPs are derived from amino acid sequences in the hydrophobic domains of natural tropoelastin, in which prolines, implicated with elastin's elasticity,[4,8−10] constitute about 12% of the total amino acid residues. Specifically, it was suggested that prolyl cis−trans isomerization may be important for the formation of a β-spiral[11] and other secondary structural motifs in elastin.[12,13]

Previously we showed that phase state and molecular architecture affect the mechanical properties of ELP molecules.[14,15] In these experiments, the measured force−extension behavior could usually be described well by a freely jointed chain (FJC) model.[16] Here we show that, when an ELP molecule is stretched for the first time or stretched after a sufficient rest time, the force−extension behavior deviates from the predictions of the FJC model at forces in the range between 200 and 260 pN. We demonstrate that the observed deviation arises from a force-induced cis−trans conformational transition of prolines, in which a stretching force applied to the ELP molecule not only elongates the molecule but also reduces the energy barrier between the proline cis and trans conformational states, thus mechanically "catalyzing" the prolyl cis−trans isomerization, which ultimately results in an increase in the $C_i^{\alpha}/C_{i+1}^{\alpha}$ atom distances (Scheme 1).[1,17] We show that a similar force-induced conformational transition occurs in poly(L-proline) but is entirely absent in poly(isoleucine). Furthermore, we support this explanation by modeling the force−extension data with an elastically coupled two-state model, using Monte Carlo (MC) simulations.[18,19]

Although SMFS by atomic force microscopy (AFM) has advanced over the past decade and has been used to characterize conformational transitions in macromolecules[20] such as poly(ethylene glycol) (PEG),[21] DNA,[16] titin,[22] and polysaccharides,[23] our research is the first to demonstrate force-induced prolyl cis−trans isomerization in polypeptides at the single-molecule level.

Materials and Methods

ELP Synthesis. The ELP 1−180 used in this study consisted of 180 pentapeptide repeats (poly(Val-Pro-Gly-Xaa-Gly)), with a total molecular weight of 71.9 kDa. It contained a leader with the amino acid sequence Ser-Lys-Gly-Pro-Gly and the amino acids Val, Ala, and Gly at the guest residue positions of the pentapeptide in a 5:2:3 ratio, respectively. The ELP was synthesized by overexpression of a plasmid-borne synthetic gene of the ELP in *Escherichia coli*.[24,25] In brief, cells harboring a plasmid that encodes for the ELP were grown in 50 mL of CircleGrow culture media (Bio101, CA), supplemented with 100 μg/mL ampicillin, with shaking at 300 rpm at 37 °C. Cell growth was monitored by the optical density (OD) at 600 nm (OD_{600}). Isopropyl β-thiogalactopyranoside (IPTG) was added to a final concentration of 1 mM at an OD_{600} of 1.0 to induce protein expression. After incubation for 3 h at 37 °C, the cells were recovered from the culture medium by centrifugation (2500*g*, 4 °C, 15 min) and resuspended in 5 mL of phosphate-buffered saline solution (PBS, 140 mM NaCl). The cells were lysed by sonication and centrifuged at 16000*g* for 20 min, and the supernatant containing the ELP was collected for purification. The ELPs were purified by inverse transition cycling, as described elsewhere.[26,27]

Sample Preparation. We prepared gold thin films with an average Au grain diameter of 30 nm on glass cover slides by thermal evaporation of a chromium adhesion layer (10 nm), followed by gold (100 nm), at a pressure of 4×10^{-7} Torr. Before deposition, the glass surfaces were cleaned for 20 min in a 1:3 (v:v) Piranha etch of H_2O_2 and H_2SO_4 at 80 °C. ***(Extreme caution must be exercised when using piranha etch! An explosion-proof hood should be used.)*** To minimize unspecific interactions between ELP and a gold surface,[28] we used a mixed self-assembled monolayer of oligo(ethylene glycol)-terminated alkanethiols (Prochimia, catalog no. TH 011-01). In this mixture, an EG_6thiol with a COOH terminal group served to attach ELP via amine coupling, while the EG_3thiol with a CH_3 terminal group served as a nonfouling background. By changing the ratio of EG_3thiol and EG_6thiol in the mixture, we were able to adjust the surface density of ELP molecules, and we settled on a ratio of 5% EG_6 and 95% EG_3. Nonspecifically adsorbed ELPs were removed by soaking the substrates in a 0.05% sodium dodecyl sulfate (SDS, Pierce) solution, followed by thorough washing with Milli-Q grade water.

In control experiment, we measured the force−extension behavior of (i) poly(L-proline) and (ii) poly(isoleucine) in water. Poly(L-proline)

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with MW = 30 kDa (Sigma Chemical Co., catalog no. P3886) and poly(isoleucine) with MW = 8.5 kDa (Sigma Chemical Co., catalog no. P3329) were dissolved in Milli-Q grade water[29] at 2 °C to a concentration of 0.1 mg/mL. Gold-coated glass slides were incubated with each protein solution for 1 h at room temperature, and force spectroscopy experiments were performed immediately after the molecules were immobilized on the gold surfaces.

Force Spectroscopy. Force spectroscopy experiments were carried out by AFM (MultiMode with Nanoscope IIIa controller, DI, Veeco) in PBS using a fluid cell attachment. Stretch−release hysteresis experiments and cantilever calibrations were performed with an MFP-3D atomic force microscope (Asylum Research). Rectangular Si_3N_4 cantilevers (TM Microscopes) were used for all experiments, and their spring constants (typically 20 pN/nm) were estimated before an experiment from the power spectral density of the thermal noise fluctuations.[30] The sensitivity of the photosensitive detector was determined from the constant compliance regime upon approach at large applied normal force. A constant pulling rate of 1000 nm/s was maintained throughout all experiments, and data were acquired with 4096 points over a distance of 1 μm in the approach and retraction path.

Data Reduction and Modeling. Force−extension curves are customarily interpreted in the framework of statistical mechanics, because a single polypeptide chain—built up from a large number of amino acids—has a large number of conformational degrees of freedom that are explored by the polypeptide on an experimental time scale that is significantly longer than that for molecular motions. Although only a single macromolecule is considered, and not a molecular ensemble, a statistical description in terms of entropy is still valid because there is enough time during a force−extension experiment for a single molecule to sample a huge number of conformations that a molecular ensemble would provide at any given moment in time. Stretching a random-coil macromolecule usually results in two force regimes. At small applied forces the molecule's response is entropic, while at large forces the molecular response becomes increasingly more enthalpic when, with increasing force, the polymer backbone is stretched and bond angles deform. At extensions much smaller than the contour length, the entropic force upon extension is readily described by the random walk statistics of the random coil. At larger extensions, the FJC model, given by a Langevin-type function, describes molecular extensibility as a function of applied force, F,[16,21]

$$x(F) = L\left[\coth\left(\frac{Fl_K}{k_BT}\right) - \frac{k_BT}{Fl_K}\right] \quad (1)$$

where L is the contour length, l_K is the Kuhn segment length, and k_B and T are Boltzmann's constant and absolute temperature, respectively. When fitting eq 1 to our experimental data, we used a weighted least-squares (WLS) approach. The method of least-squares assumes a normal distribution of errors; however, for a typical force−extension curve measured with AFM, the assumption of constant variance is violated (see Figure A.1, Supporting Information). Observations at low forces are subject to significantly greater fluctuation in separation than those at higher forces; i.e., the variance $\sigma^2(F)$ decreases with increasing force. When σ^2 is not constant, maximum likelihood estimates are obtained by a WLS minimization. Weight factors used in the least-squares when fitting a polymer elasticity model were obtained from a Langevin or a von Mises−Fisher distribution.[31] Force−extension data were analyzed with a custom Matlab program.

Results

Cis−Trans Isomerization in ELPs Measured by Atomic Force Spectroscopy. In our data analysis procedure, we selected only those force curves that showed a single force−extension event. The contour lengths obtained by fitting the FJC model (eq 1) to force−extension data were all shorter than the theoretically possible contour length of about 318 nm for a single ELP1−180. When a molecule was extended for the first time (or after sufficient rest time, >1 s), we observed a small (order 1.5−3.5 nm) step increase in elongation, deviating from the predictions of a FJC model at forces in the range between 200 and 260 pN. We found that this deviation occurs in good (PBS) as well as poor solvents (PBS + 1.5 M NaCl), suggesting that it is independent of the solvent conditions and phase state of the ELP (Figure 1).

Deviations of about 1.5 and 3.2 nm can be seen more clearly in Figure 2 and the corresponding separation residuals plots (inset of Figure 2), which were produced by subtracting the predicted extension (obtained from the FJC model fit to force−extension data below 200 pN) from the experimentally measured

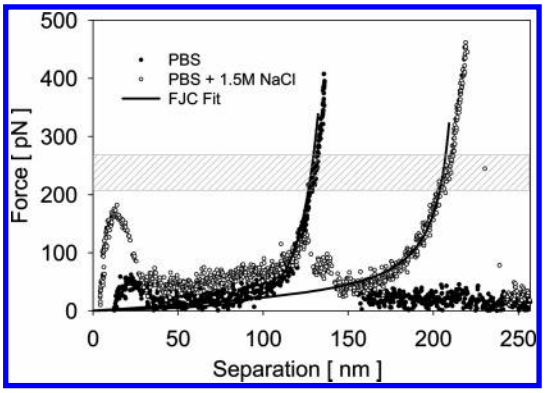

Figure 1. Representative force−extension curves for ELP1−180 in good solvent (PBS) and in poor solvent (PBS + 1.5 M NaCl) with corresponding FJC fits. FJC fits were obtained in a force window below 200 pN (fit parameters in PBS, $l_K = 0.38$ nm and $L = 135$ nm; in PBS + 1.5 M NaCl, $l_K = 0.40$ nm and $L = 215$ nm). A small elongation transition occurred for ELP in both solvents at forces in the range between 200 and 260 pN (gray band).

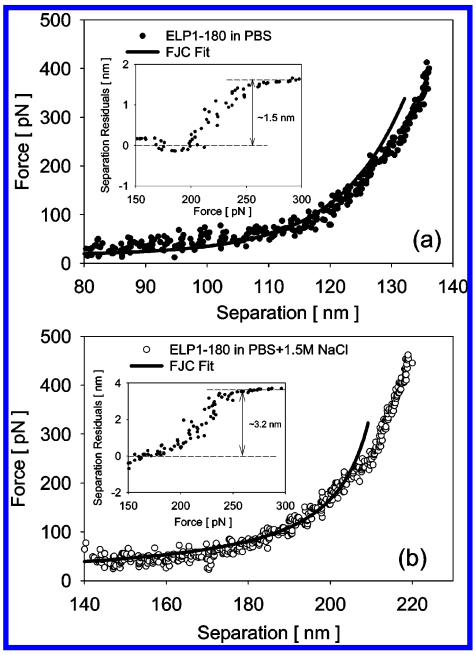

Figure 2. Zoomed-in view of the two force−extension curves in Figure 1, showing the elongation transition in the 200−260 pN force range more clearly for ELP1−180 (a) in PBS and (b) in PBS + 1.5 M NaCl. Insets: separation residuals.

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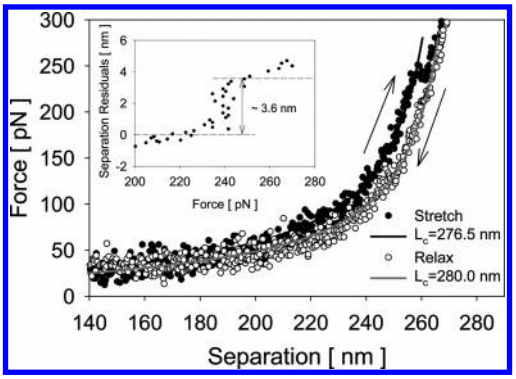

Figure 3. Typical hysteresis experiment for ELP1−180 in PBS showing, at a pulling speed of 1000 nm/s, a deviation between extension and relaxation pathways that occurred at a force of about 240 pN. The solid lines show the FJC fits to the extension ($l_K = 0.35$ nm, $L = 276.5$ nm) and relaxation ($l_K = 0.37$ nm, $L = 280.0$ nm) data. Inset: separation residuals.

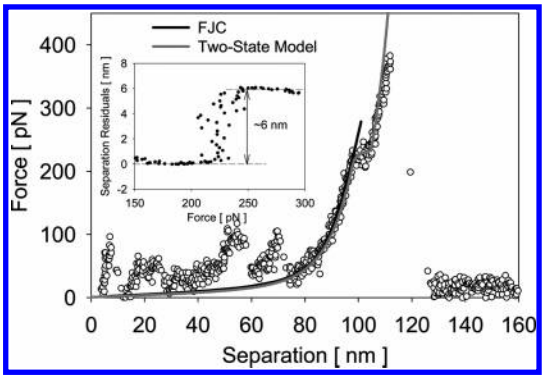

Figure 4. Representative force−extension curve for poly(L-proline) and corresponding fits of a FJC model (black line) and a MC simulation (gray line). The inset shows the separation residuals.

extension in the range between 150 and 300 pN. Figure 2 also shows that the molecular elongation is larger for the ELP with a longer tethered length. The elongations predicted from separation residual plots are in good agreement with the expected deviations due to prolyl cis−trans isomerization, as discussed in detail below.

Figure 1 also shows that stretching in PBS + 1.5 M NaCl results in significantly larger nonspecific adhesion forces at small extensions when compared with stretching in PBS. This observation indicates a more collapsed, potentially entangled, hydrophobic state of the ELP upon increase in solution ionic strength and is consistent with previous studies of the ELP phase transition.[26,32]

To investigate the refolding kinetics of the observed deviation, we conducted stretch−relaxation experiments. Figure 3 shows that the conformational transition was irreversible on the time scale of our experiment (~1 s for each stretch/relaxation cycle). The deviation between stretch and relaxation pathways occurred at a force of about 240 pN and can be seen clearly in the separation residual plot (inset Figure 3).

Comparison of Theoretically Predicted and Observed Elongation. Next we show that the observed stretch-induced elongation deviation is consistent with that expected from a simple consideration of the number of prolines that contribute to the cis−trans isomerization in an ELP force−extension experiment. There are a total of 180 prolines in the amino acid sequence of ELP1−180, and if we assume that the cis isomers in ELP1−180 contribute to 12% of the total number of prolines[1,2] and that the molecular elongation due to prolyl cis−trans isomerization is 0.2 nm, then a maximal possible elongation of 4.3 nm is expected for a single ELP1−180 molecule. In a typical force spectroscopy experiment, however, an AFM cantilever tip attaches randomly along the length of the ELP backbone. At an extension ratio of about 70%, as shown in Figure 2b, only a fraction of prolines will undergo the conformational transition, and the expected deviation will be about 2.6 nm. This is reasonably consistent with our separation residual plot (inset of Figure 2b), which shows an elongation of 3.2 nm.

The thermally driven conversion from the trans into the cis isomeric state is slow due to the large activation barrier for the cis−trans isomerization. This was shown in our hysteresis experiments, where a single ELP molecule was extended and immediately relaxed, without rupture (Figure 3). The elongational deviation due to prolyl cis-to-trans isomerization occurred only in the stretching pathway but was absent during relaxation, indicating a nonequilibrium process on the time scale of the experiment (<1s). A fit of the experimental force−extension data with a FJC model (eq 1) showed a reasonable increase in contour length (~3.6 nm), due to prolyl cis-to-trans isomerization.

Stretch−relaxation experiments with an AFM are difficult to perform and likely will not reveal further detail on the refolding kinetics due to the small zero-force trans-to-cis transition rate ($\beta_0(F) = 8.2 \times 10^{-4}\ s^{-1}$, see below) that decreases further upon application of a mechanical stretching force. However, determination of the pulling rate dependence of the prolyl cis-to-trans isomerization force by dynamic force spectroscopy may provide a means to characterize the unfolding and cis-to-trans kinetics so that the zero-force cis−trans rate and the transition potential width can be obtained.

Control Experiments with Poly(L-proline) and Poly(isoleucine). To test our hypothesis of the force-induced cis−trans prolyl isomerization, we performed force spectroscopy experiments with surface-grafted poly(L-proline) (PLP). While the force−extension behavior of PLP was similar to that of ELP, the force-induced elongation due to cis-to-trans prolyl isomerization was, as expected, significantly larger than that observed for ELP (Figure 4). A typical PLP molecule in our experiments is composed of approximately 260 prolines (MW = 30 kDa). IR studies of the cis-to-trans transition in water suggest that both cis and trans isomers in PLP are populated; however, the exact ratio of cis to trans isomers was not indicated in that study.[29] The observed force-induced elongation in our experiments suggests that about 14% of the prolyl bonds contained in a single PLP molecule were isomerized from the cis into the trans state during stretching.

As a negative control, we selected poly(isoleucine) (MW = 8.5 kDa) instead of simpler amino acids, such as poly(alanine) or poly(glycine), because only poly(isoleucine) was commercially available with a high molecular weight in the same range as the ELP. As expected, in force−extension experiments with poly(isoleucine), the characteristic "signature" of the cis−trans prolyl isomerization was absent (Figure 5).

A gradually increasing deviation, observed above about 300 pN (Figure 5), arises from the limitation of the FJC model.

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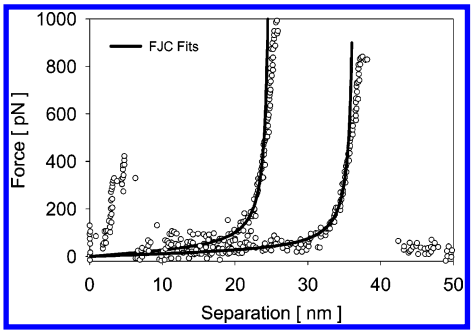


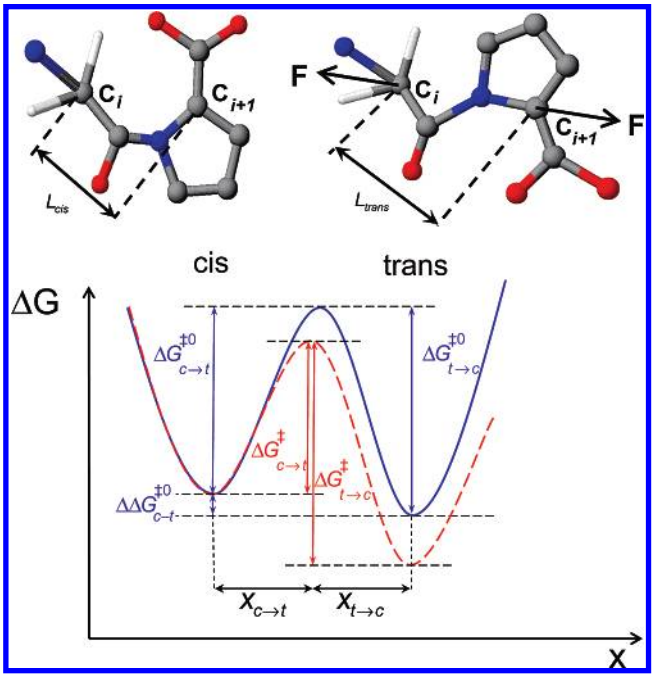
Figure 5. Representative force−extension curves for poly(isoleucine) and corresponding FJC fits (solid lines). Bond-angle deformations become important above 300 pN, and the simple FJC model fails to fit the data.

Above ~300 pN, the molecule starts deforming elastically, and its force−extension behavior is better described by the extended FJC model, which accounts for enthalpic contributions due to bond angle torsion and bending.[21,24]

Modeling the Cis−Trans Isomerization. To further validate our hypothesis of force-induced prolyl cis−trans isomerization, we modeled the force−extension behavior with an elastically coupled two-state system.[19] This model has been applied previously to simulate unfolding of, e.g., titin[18,19] and dextran.[19] Two necessary inputs for the simulation are the length change of the molecule associated with the cis−trans isomerization and the difference between the activation free energy minima corresponding to the cis and trans states of proline. The height of the activation free energy barrier and the length gain upon conversion of cis proline into its trans isomer depends on the polypeptide length and amino acid sequence.[1] As shown in Scheme 2, an extensional force applied to an ELP molecule lowers the activation free energy barrier between the proline cis and trans isomeric states, increases the activation free energy barrier between the trans and cis states, and thus effectively increases the probability that a prolyl cis-to-trans isomerization will occur.

In accordance with previously published data, we chose the difference between the activation free energy minima corresponding to the cis and trans states of proline to be $\Delta\Delta G^{\ddagger 0}_{c-t} \cong$ 5 kJ/mol, and we chose the total elongation associated with cis−trans isomerization to be 0.2 nm, equal to the sum of the barrier widths, i.e., $d_{trans} - d_{cis} = x_{c\to t} + x_{t\to c}$.[1,3,17] In addition, we assumed that the barrier height changes linearly with the reaction coordinate. A linear free energy relationship in a two-state model can then be described by $\Delta G^{\ddagger}_{c\to t} = \Delta G^{\ddagger 0}_{c\to t} - \Phi\Delta\Delta G^{\ddagger 0}_{c-t}$, where the fraction Φ determines the position for the top of the energy barrier, in our case $\Phi = 1/2$ since $x_{c\to t} = x_{t\to c}$ (see Scheme 2). We used the experimental extension rate (1000 nm/s), the fitting parameters for Kuhn segment length, and the contour length of the inverse FJC model to generate the force input for the MC simulation. The only adjustable parameter in the MC simulation was the height of the activation free energy barrier. We obtained a value of $\Delta G^{\ddagger 0}_{c\to t} \cong$ 60 kJ/mol by matching the predictions of the MC simulation to the measured force range over which the cis−trans isomerization occurred in the experiments. This value for the cis−trans activation energy barrier is in good agreement with (i) the range of values (60−80 kJ/mol) reported by in a review by Dugave et al.[1] for model peptides, (ii) values reported by Brandts et al.,[3] who studied the effect of cis−trans isomerization on the refolding kinetics of small proteins, and (iii) values from studies of enzymatic catalysis of cis−trans isomerization.[33,34]

Scheme 2. Schematic of the Free Energy Landscape of the Prolyl Cis−Trans Isomerization, Showing the Effect of Force on the Transition[a]



[a] At equilibrium (solid line), the cis and trans conformational states of proline in ELP are separated by activation free energy barriers $\Delta G^{\ddagger 0}_{c\to t}$ and $\Delta G^{\ddagger 0}_{t\to c}$, respectively, where $\Delta\Delta G^{\ddagger 0}$ reflects the energy difference between these two barriers. Application of a stretching force lowers the activation energy barrier for the cis-to-trans isomerization ($\Delta G^{\ddagger}_{c\to t}$) and raises the barrier for trans-to-cis isomerization ($\Delta G^{\ddagger}_{t\to c}$) (dashed line). The barrier width is symmetric, with $x_{c\to t} = x_{t\to c}$, and assumed to be independent of the applied force.

Figure 6 shows the remarkably good match between the predictions of the MC simulation of the two-state system and the experimentally observed deviation in the measured force−extension curve (see Supporting Information for further details on the MC simulation).

Using $\Delta G^{\ddagger 0}_{c\to t} \cong$ 60 kJ/mol and a barrier width of 0.1 nm, we estimated the equilibrium constant at zero force, $\alpha_0(F)/\beta_0(F) = 10.2$ ($\alpha_0(F) = 8.4 \times 10^{-3}$ and $\beta_0(F) = 8.2 \times 10^{-4}$), and at the force of 220 pN, $\alpha(F)/\beta(F) = 5.3 \times 10^5$ ($\alpha(F) = 2.5$ and $\beta(F) = 4.7 \times 10^{-6}$). This significant increase in the equilibrium constant suggests that, with increase in force, the cis-to-trans isomerization is strongly favored over the backward trans-to-cis transition.

We also performed a MC simulation of a two-state system for poly(L-proline). As with ELP, we took the length difference between cis and trans conformations of proline to be 0.2 nm, and we chose to use the same activation free energy of ~60 kJ/mol that we found previously for cis-to-trans isomerization in ELP. Figure 5 shows that the simulation predictions closely match the experimentally measured force−extension behavior.

A Kinetic Pathway for Cis−Trans Isomerization. Two possible kinetic pathways for the isomerization reaction under external force are shown in Scheme 3. In the absence of an

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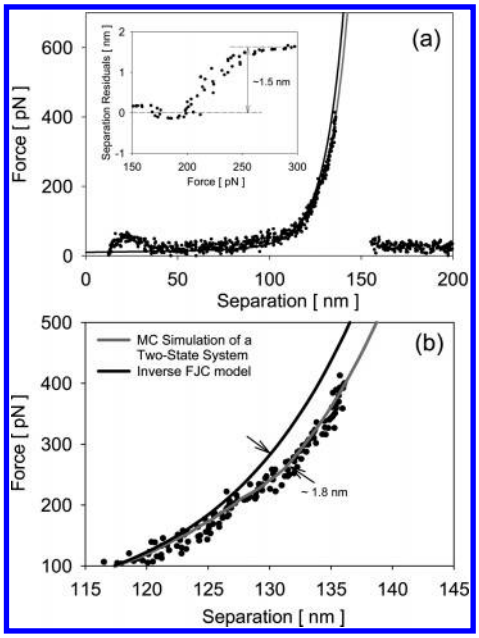
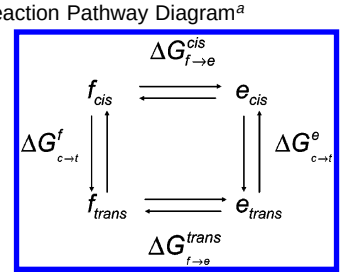


Figure 6. (a) Force−extension data fitted with an inverse FJC model (black line) and predictions of a Monte Carlo simulation of an elastically coupled two-state system (gray line). The inset shows separation residuals. (b) Magnified portion of the force−extension curve in (a).

Scheme 3. Reaction Pathway Diagram[a]



[a] $\Delta G^{f}_{c\to t}$ and $\Delta G^{e}_{c\to t}$ are the Gibbs free energies for cis−trans isomerization in the folded state (no applied stretching force) and extended state (applied force), respectively. $\Delta G^{cis}_{f\to e}$ is the energy required to stretch the fragment of the molecule that contains cis proline from the folded state into the extended state, and $\Delta G^{trans}_{f\to e}$ is the energy required to stretch the fragment of the molecule that contains trans proline from the collapsed state into the extended state.

applied force, ELP is in a "folded" state containing proline in the cis and trans conformations, denoted here as f_{cis} and f_{trans}. Molecular stretching leads to "extended" states of ELP with prolines in the cis (e_{cis}) and trans (e_{trans}) conformations.

Scheme 3 suggests that cis−trans isomerization can occur along two possible energetic pathways:

$$f_{cis} \leftrightarrow e_{cis} \leftrightarrow e_{trans} \quad \text{(i)}$$

$$f_{cis} \leftrightarrow f_{trans} \leftrightarrow e_{trans} \quad \text{(ii)}$$

Pathway (i), however, is favored since cis−trans prolyl isomerization is catalyzed by the stretching force, as shown above.

Discussion

Protein folding often involves the rate-limiting cis-to-trans isomerization of prolines.[2,3] Because of the high activation energy barrier,[2] this isomerization occurs only slowly at equilibrium, where in the absence of structural constraints, both cis and trans isomeric states are significantly populated since the difference in the activation free energy barriers between the two states is small.[2,5] To better understand the energetics of the prolyl cis−trans isomerization, we can depict the energy change along the reaction coordinate (see Scheme 2). In thermal equilibrium, two free energy minima, corresponding to the cis and trans states, are separated by the barriers $\Delta G^{\ddagger 0}_{c\to t}$ and $\Delta G^{\ddagger 0}_{t\to c}$, respectively. In the absence of an externally applied force, there is an equilibrium concentration of proline cis and trans isomeric states in each ELP molecule. A stretching force decreases the free energy barrier for the cis-to-trans isomerization (dashed red line in Scheme 2) and increases the activation free energy barrier between the trans and cis states, thus effectively increasing the probability that a prolyl cis-to-trans isomerization will occur.

As every ELP molecule contains a large number of prolines (e.g., 180 prolines for the ELP studied here), an ensemble of cis and trans isomers must be considered. The cis−trans isomerization in this ensemble, when modeled by a two-state system, does not explicitly include chemical cooperativity; i.e., the isomerization of proline from the cis into the trans state is independent of the conformational state of any other proline in the same molecule. However, some level of cooperativity is implicit by applying a MC simulation of a two-state system, since the number of prolines in the cis form at each step of the simulation influences the probability for cis−trans isomerization (see Supporting Information, eq A.4).

In the interpretation of the predictions of the two-state model simulations, we assumed that, on the experimental time scale, the trans-to-cis prolyl isomerization is sufficiently slow; i.e., the number of cis and trans conformational states reflects an inequilibrium. The validity of this assumption is supported by the hysteresis observed in our stretch−relaxation experiments (Figure 3), where isomerization of prolines occurs only on the extension path. After the molecule is relaxed, prolines do not return to their equilibrium conformation immediately. Furthermore, the elongation mechanism was exhausted after repeated stretching of the same molecule at the typical stretching rate of 1000 nm/s.

Our MC simulations did not aim to provide an exact quantitative treatment of the prolyl cis-to-trans isomerization energetics but rather tried to capture the experimentally observed deviation from FJC behavior. A precise estimate of energy barriers for the prolyl cis-to-trans isomerization can be problematic for several reasons. First, a statistical analysis of structural changes of various proteins during peptidyl−prolyl isomerization shows that a conversion from the cis into the trans conformation induces an elongation of 1.3 ± 0.6 Å for some proteins, while others exhibit elongations of 3.0 ± 2 Å.[17] In our calculations, we assumed an intermediate value of 2 Å. Second, the free energy barrier width of the elastically coupled two-level system used in the simulation was assumed to be symmetric and independent of force. Third, the isomer-specific atom distances can produce both symmetric and asymmetric distributions for the N-terminal and C-terminal segments flanking the proline residue. We assumed that isomerization affects only proline's nearest neighbor; however, an effect can persist up to four residues away from the proline.[17] Fourth, the energetic parameters reported in the literature[2,34,35] and used here were mostly determined in spectrophotometric assays on

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recognition peptides and were not specifically obtained for the ELPs used in our experiments. Fifth, in determining the force-dependent unfolding rates, we used the approach developed by Bell.[36] This approach is simpler than the more elaborate treatment developed by Evans, who studied bond dynamics under applied forces.[37] In calculation of force-dependent rate constants, Evans's approach accounts for viscous friction, and thus the prefactor in the rate equations is no longer a constant but now also depends on force. Finally, in modeling our results, we assume that the stretching force is applied along the backbone of the protein; however, during force−extension measurements, a molecule is likely not aligned perfectly along the stretching direction. This misalignment contributes to the range of forces (i.e., 200−260 pN) over which we observed the deviation from the polymer elasticity model.

The measured separation residuals yielded ELP elongations during cis-to-trans isomerization that were typically larger than the theoretical estimate. A likely explanation for this discrepancy arises from the use of an average literature value for the length gain associated with the prolyl cis-to-trans isomerization in ELP and our estimate of the fraction of prolines in the cis state. This fraction can potentially vary from 10% up to 30%, depending on the amino acid sequence and type of the solvent.[1,2]

Conclusions

Using single-molecule force spectroscopy, we showed for the first time that force can induce the prolyl cis-to-trans isomerization in proline-containing polypeptides. We were able to detect this subtle, force-induced conformational change by measuring the force−extension behavior of elastin-like polypeptides, which are composed of many prolines that can undergo isomerization from the cis into the trans state under applied force. A simple Monte Carlo simulation, treating the prolyl cis−trans isomerization as a two-state system, confirmed our measurements and yielded information about the reaction energetics and the rate constants. Our approach thus provides, for suitable proteins (i.e., proteins with sufficient numbers of proline residues), an alternative means to study the energetics and possibly the kinetics of prolyl cis−trans isomerization.

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Supporting Information Available: Details on modeling the prolyl cis−trans isomerization with an elastically coupled two-state system and analyzing the force−extension curves. This material is available free of charge via the Internet at http://pubs.acs.org.

JA070147R

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