Proline Isomerization Regulates the Phase Behavior of Elastin-Like Polypeptides in Water

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ABSTRACT: Responsiveness of polypeptides and polymers in aqueous solution plays an important role in biomedical applications and in designing advanced functional materials. Elastin-like polypeptides (ELPs) are a well-known class of synthetic intrinsically disordered proteins (IDPs), which exhibit a lower critical solution temperature (LCST) in pure water and in aqueous solutions. Here, we compare the influence of cis/trans proline isomerization on the phase behavior of single ELPs in pure water. Our results reveal that proline isomerization tunes the conformational behavior of ELPs while keeping the transition temperature unchanged. We find that the presence of the cis isomers facilitates compact structures by preventing peptide−water hydrogen bonding while promoting intramolecular interactions. In other words, the LCST transition of ELPs with all proline residues in the cis state occurs with almost no noticeable conformational change.

1. INTRODUCTION

Stimulus-triggered polypeptides are involved in a wide range of biological processes. For example, the liquid−liquid phase separation of intrinsically disordered proteins (IDPs) is found to contribute to the formation of membraneless organelles,1,2 and the self-assembly of IDPs is associated with numerous human diseases3,4 including neurodegenerative disorders, cancer, and amyloidoses. Synthetic polymers that exhibit phase transitions also have broad applications ranging from biomedical applications5−9 to polymer materials design.10−15 Therefore, the microscopic understanding of the phase behavior of stimuli responsive polymers is crucial for the optimized future applications.14,16

Elastin-like polypeptides (ELPs)16,17 are synthetic peptide-like polymers with pentapeptide repeat sequences Val-Pro-Gly-Xaa-Gly (VPGXG), where the guest residue Xaa can be any amino acid except proline. They typically exhibit a lower critical solution temperature (LCST) phase behavior in aqueous solution, with an expanded-to-collapsed conformational transition. The transition temperature Tl of ELPs is tunable and depends on the peptide sequence, the chain length,18 and a number of external stimuli, such as changes in pH,19 ion concentration,20 and pressure.21 ELPs are proline-rich peptides; however, the effects of proline isomerization on their phase behavior remain unclear. Proline is the only amino acid with a cyclic side group; i.e., its nitrogen atom is linked to two carbon atoms, forming a five-membered ring (see Figure 1). This unique structure stabilizes both cis and trans isomers. While the Gibbs free energy difference between the two proline isomers is only ~2 kBT,22,23 their transition barrier is rather high, ~30−32 kBT.22,24 Therefore, proline isomerization is a fairly slow rate-limiting process,25 which is important in understanding protein folding kinetics.26 In nature, the trans isomer is dominant in Xaa−Pro

Figure 1. Schematic representation of (VPGVG)30 with trans or cis proline isomers. The backbone residues which restrict the ω dihedral angle of the Val−Pro amide bonds are marked in red; ω = 180° for the trans isomer, while it is 0° for the cis isomer.

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peptide bonds with a trans-cis ratio\textsuperscript{7} of about 88:12, in excellent agreement with a direct Boltzmann weight energy based estimate. However, one can enhance the cis isomer content in a number of ways including replacing a proline with a pseudoproline named \textit{ΨPro},\textsuperscript{25} using proline isomerase assay\textsuperscript{22} or the C(4)-position substituent,\textsuperscript{28} and also perhaps by ultraviolet photodissociation.\textsuperscript{29}

In this work, we focus on the effect of proline isomerization on the phase behavior of ELPs using all-atom simulations. We consider an ELP sequence of (VPGVG)\textsubscript{n} with four different cis proline compositions: (i) all proline residues are in the trans state \( P_{\text{cis}} = 0 \); (ii) half of the proline residues are in the cis state \( P_{\text{cis}} = 0.5 \), and they are either organized in two blocks \( \ldots\text{ccc} \ldots\text{ttt} \ldots \) or (iii) ideally mixed, \( \text{ctctctctct ctctctctct ctctctctct} \); and (iv) all of the proline residues are in the cis state \( P_{\text{cis}} = 1.0 \). Note that these are model sequences to best isolate the effect of the cis isomers. To simplify the notation, these four cases will be denoted as all-trans, hs-cis, hm-cis, and all-cis, respectively, in the following text. Because of the high energy barrier, the trans/cis composition remains constant during the course of the simulation. Our results show that proline isomerization plays an important role in tuning the conformational behavior of ELPs in water while keeping \( T_j \) unchanged. The presence of the cis isomers facilitates rather compact structures of the peptide. These structures remain largely stable in the temperature range studied, because of enhanced intramolecular and reduced peptide-water hydrogen bonds. The compactness of the peptide is a function of both the percentage and the position of cis isomers. The more cis isomers and the more distributed they are along the sequence, the more compact the chains are.

Our study reveals that the conformational behavior of ELPs and other proline-rich peptides can be regulated by proline isomerization while keeping their transition temperature unchanged. To demonstrate this most clearly, we especially focus on the all-trans and all-cis cases.

2. METHODS

The all-atom simulations of (VPGVG)\textsubscript{n} were performed using the GROMACS molecular dynamics (MD) package,\textsuperscript{30} where the initial structures were prepared with the PyMOL package\textsuperscript{31} (see Figure S1a,b in the Supporting Information). The MD simulations were performed in the NPT ensemble, using the CHARMM36m force field\textsuperscript{32} together with the TIP3P water model.\textsuperscript{33} The pressure was kept at 1 bar using the Parrinello–Rahman–Andersen barostat\textsuperscript{34} with a coupling constant of 2 ps, and the temperature of the system was kept constant by a velocity rescaling thermostat\textsuperscript{35} with a coupling constant of 1 ps. The electrostatic interactions were simulated using the particle mesh Ewald (PME) algorithm.\textsuperscript{36} The cutoff of the electrostatic and van der Waals interactions was set to 1.4 nm. We used the LINCS algorithm\textsuperscript{37} for bond constraints. The equations of motion were integrated using the leapfrog integrator with a time step of 2 fs.

The chosen ELP with sequence (VPGVG)\textsubscript{n} is a relatively well studied system.\textsuperscript{38,39} Its transition temperature was computationally estimated\textsuperscript{38} (here the atomistic simulations were performed using \textit{Amber} 11 with ff99SB force, which might lead to slightly different transition temperatures) as \( T_j = 307.5 \pm 2.5 \) K. For such a short chain, one cannot expect a sharp phase transition in the simulations. Therefore, we considered the simulation temperature range from \( T = 280 \) to \( 320 \) K to take into account the region around its LCST transition. In our simulations, the peptide was hydrated in a 10 nm \( \times \) 10 nm \( \times \) 10 nm box with 32,177 water molecules. We used the replica exchange molecular dynamics (REMD) to enhance the sampling of the peptide in the all-trans case around its transition temperature. In total, there are five temperature replicas ranging from 300 to 310 K, and the exchange of replicas was attempted every 2 ps. The REMD simulations lasted for 1 \( \mu \)s. In other cases, we generated two independent trajectories for each temperature, and these trajectories covered a time of 1 \( \mu \)s.

The geometry of the peptide with cis or trans proline isomers was characterized by measuring the \( \omega \) dihedral angle of the Val–Pro amide bonds and the effective backbone length \( L = \sum_{i=2}^{N} | \mathbf{r}_i - \mathbf{r}_{i-1} | \). Here, \( \mathbf{r}_i \) corresponds to the position of the \( C_a \) atom of the \( i \)th residue along the backbone of (VPGVG)\textsubscript{n} and \( N = 150 \) is the total number of residues. Because of the high energy barrier between the cis and trans states, no cis/trans transition was observed in the course of our simulations for a given case. The dimension of the peptide was characterized by the gyration radius \( R_g = \left( \frac{1}{2N} \sum_j (\mathbf{r}_j - \bar{\mathbf{r}})^2 \right)^{1/2} \), the end-to-end distance \( R_E = \sqrt{\sum_j (\mathbf{r}_j - \bar{\mathbf{r}})^2} \), the solvent-accessible surface area (SASA)\textsuperscript{40}, and the single-chain backbone structure factor \( S(q) = \left( \frac{1}{N} \sum_{i=1}^{N} \exp(iq \cdot \mathbf{r}_i) \right)^2 \), where \( q \) is the wave vector.

Here, the calculation of \( S(q) \) is based only on the C\textit{a} atoms. The secondary structural content of the peptide including the propensity of \( \beta \)-sheet and \( \alpha \)-helix was estimated by the DSSP algorithm, which assigns secondary structures based on the backbone hydrogen bonds using an electrostatic model.\textsuperscript{41} We also performed a hydrogen bonding analysis by counting both the number of hydrogen bonds (H-bonds) between the peptide and water molecules \( N_{pw} \) and the number of the intramolecular H-bonds within the peptide \( N_{pp} \). Additionally, we counted the H-bonds formed between proline and the non-proline residues \( N_{pro,op} \) to characterize the effects of cis/trans proline isomerization. H-bonds are estimated using the standard GROMACS subroutine; i.e., an H-bond exists if the donor–acceptor distance is \( \leq 0.35 \) nm and the acceptor–donor-hydrogen angle is \( \leq 30^\circ \). To characterize the density of water molecules within distance \( r \) from the peptide, we calculated the radial distribution function between the backbone of the peptide and the oxygen atoms on the water molecules \( g_{pw}(r) \).

3. RESULTS AND DISCUSSION

To characterize the internal structure (backbone orientation) of (VPGVG)\textsubscript{n} for the four distinct cis proline compositions considered, we analyze the dihedral angles, \( \omega \), of the Val–Pro amide bonds and the effective backbone lengths \( L \). The distribution of \( \omega \) at \( T = 280 \) K is shown in Figure 2a. Note that the \( \omega \) distribution remains the same with temperature; see Figure S1c,d. We find that the average value of \( \omega \) is \( 170^\circ \) for the trans Val–Pro bonds, while it is \( -15^\circ \) for the cis bonds, roughly consistent with the ideally expected difference of \( 180^\circ \) between trans and cis isomers. This small deviation in \( \omega \) is expected because of the local bending and packing interactions within a molecule. In the cases of hs-cis and hm-cis, the distribution of \( \omega \) in the region with trans isomers is the same as...
that from the all-trans case, while that with cis isomers is the same as that for the all-cis case.

Figure 2b shows the distribution of $L$ along with the average values ($\langle L \rangle = 56.2, 54.8, 54.8, \text{and} 53.4 \text{ nm}$ in the cases of all-trans, hs-cis, hm-cis, and all-cis, respectively). The difference of ($L$) between the all-trans and all-cis cases is $\Delta L = \langle L_{\text{cw}} \rangle - \langle L_{\text{cm}} \rangle = 2.8 \text{ nm}$, which gives an average elongation of $\sim 0.1 \text{ nm}$ per proline. The elongation has also been observed experimentally, which indicates a similar backbone change of cis-to-trans isomerization, as shown in our simulations. The results of $L$ and $\omega$ provide a detailed geometric picture of the peptide; i.e., its internal structure with the trans isomers is different than that with the cis isomers.

In Figure 3a, we show the effects of proline isomerization on the gyration radius $R_g$ of the system as a function of temperature. It can be seen from the all-trans data that the peptide shows a well-defined expanded-to-collapsed transition upon an increase of temperature; the detected LCST transition temperature (approximate inflection point of the curve) is around $T_l \approx 280 \text{ K}$. The obtained $T_l$ is in very good agreement with experiments, which found $T_l = 299 \text{ K}$ for the sequence (VPGVG)$_{30}$.

At the other extreme, the all-cis case, $R_g$ is nearly independent of temperature; i.e., no LCST-like transition signature in $R_g$ is observed. In the mixed cases, the size of the peptide is in between the former two. Additionally, the difference between $R_g$ of hs-cis and hm-cis illustrates how the structure of the peptide is affected by the sequence of the cis isomers along the backbone. In particular, $R_g$ of hs-cis follows a transition similar to the all-trans case albeit more attenuated (half is collapsed and half remains expanded when $T < T_l$), while that of hm-cis is further reduced. Note that cis isomers may contribute up to 12% of Val-Pro peptide bonds in nature. Thus, we have considered an additional system with $P_{\text{cis}} = 0.1$, close to the natural cis content. As shown in Figure S2, 10% cis content leads to a $5-13\%$ decrease of $R_g$ at temperatures below $T_l$ depending on the trans/cis sequence, while the transition temperature is not affected.

To characterize the local interactions of the peptide, we also estimate the propensity of secondary structure formation. For the sequence (VPGVG)$_{30}$, we expect it to form $\beta$-sheets (it has two valine residues in each pentapeptide) but not $\alpha$-helices (proline and glycine are known to prohibit helix formation). It is indeed the case in the all-trans case but not in the all-cis case, which also has no $\beta$-sheets. Figure 3b presents the results of $f_{\beta}$, the fraction of $\beta$-sheets formed by connecting the adjacent $\beta$-strands laterally with hydrogen bonds ($f_{\beta}$ is obtained by the DSSP algorithm). We find that $f_{\beta}$ exhibits a similar trend as a function of temperature as $R_g$ in the four systems. In the all-trans case, $f_{\beta}$ decreases monotonically from $\sim 20$ to $\sim 5\%$ as $T$ increases from 280 to 320 K. The temperature induced decrease in $f_{\beta}$ is due to the fact that less $\beta$-strands can be laterally placed to form $\beta$-sheets as the chain becomes more compact. A similar pattern is seen in the hs-cis case with smaller values of $f_{\beta}$ for $T < T_l$. A closer look reveals that the decrease is mostly coming from the region with trans isomers (see Figures S3 and S4). This observation clearly shows that the presence of the cis isomers sterically hinders the formation of hydrogen bonds between local segments, which explains why $f_{\beta}$ is nearly zero at all temperatures in the hm-cis and all-cis cases. In conclusion, we observe that $T_l$ of the ELP seems independent of the percentage and position of the cis isomers (see the vertical line in Figure 3a), yet the amplitude of the $R_g$ signature and the secondary structural content are strongly dependent on the cis composition. For the all-cis case, the $R_g$ signature on the single channel level almost vanishes, requiring further consideration.

The global conformational dynamics of the ELP is also well characterized by the backbone structure factor $S(q)$. $S(q)$ for the all-trans and all-cis cases at $T < T_l (T = 280 \text{ K})$ and $T > T_l (T = 320 \text{ K})$ are presented in Figure 3c,d. Results for other temperatures are shown in Figure S5. The $S(q)$ data show that at $T = 280 \text{ K}$ the all-cis chain assumes a globular state ($q^{-4}$ scaling), while the all-trans chain more closely resembles a random walk structure ($q^{-2}$) at $q$ values below about $2 \text{ nm}^{-1}$ with a more compact regime on shorter length scales. On smaller scales above $q \approx 4 \text{ nm}^{-1}$, $S(q)$ is very similar in both cases. At $T = 320 \text{ K}$, the all-trans chains collapse into an even more pronounced globular structure compared to the all-cis case, as demonstrated by Figure 3d ($S(q)$ of the all-cis chain remains essentially unchanged from $T < T_l$ to $T > T_l$). Data for the hs-cis and hm-cis cases interpolated between these two extremes are shown in the Supporting Information. The power laws indicated by dashed lines are used to guide the eye. Clearly, the peptide behaves roughly similar to a short polymer chain in between the $\Theta$ and the collapsed state in the all-trans case, and it becomes significantly more compact from the outset as $P_{\text{cis}}$ increases. At high $q$ when $q > \frac{2\pi}{L} \sim 3 \text{ nm}^{-1}$, the scaling of $S(q)$ transitions to $q^{-1}$ in all cases, where the peptide
behaves essentially like a rigid rod. These data agree well with the observations obtained from $R_g$ and $f_p$. Moreover, the obtained Kuhn length $l_k \sim 2 \text{ nm}$ (the length of 5–6 residues) agrees with our previous simulation and the experimental results for ELPs. We also calculated the hydrodynamic radius $R_h$ of (VPGVG)$_{30}$ data are shown in Table S1 for reference. For ideal chains, one can estimate the end-to-end distance $R_e$ of the chain directly by the Kuhn length as $R_e^{(3)} = l_k$. Using $L = 56.2 \text{ nm}$ (see Figure 2) and $R_e$ shown in Table S1 is a clearly too small value for $l_k$, revealing significant deviations from a Gaussian structure. In general, the data presented here agree quite well with experimental data obtained from dynamic light scattering (for details of the different radii, we refer to the Supporting Information). The deviations from the classical polymer picture may be due to the fact that the ELP chain has a fraction of $\sim 20\%$ of $\beta$-sheets in the all-trans case at $T < T_c$. Nevertheless, the applied model can successfully catch the LCST transition of the ELP (Figure 3) and can distinguish between the cis and trans proline states (Figure 2). Above the LCST, $S(q)$ displays a $q^{-4}$ scaling in all considered cases, which means that the peptide is collapsed regardless of the cis content. Note that, although the shape parameters $S(q)$ and $R_g$ in all considered cases are alike at $T > T_c$, the internal structure (which can be characterized by the $\omega$ dihedral angles of the Val–Pro amide bonds and the effective backbone lengths $L$) of the peptide remains very different, as shown in Figure 2.

The LCST phase transition (the expanded-to-collapsed transition upon increase of temperature) is entropy driven, i.e., dominated by the translational entropy gain of the water molecules upon collapse of the peptide around $T > T_c$. The amount of released water molecules can be visualized by the radial distribution function. As shown in Figure 4a, $g_{pw}(r)$ of (VPGVG)$_{30}$ has three peaks within $r \leq 1.0 \text{ nm}$ (note that the correlation length of water molecules is less than $2.0 \text{ nm}$ in the considered systems). We find that the height of the peaks decrease as $T$ increases, because the peptide becomes more compact (see Figure 3a). However, in the all-trans case, a jump of $g_{pw}(r)$ around $T_c$ is observed, while, in the all-cis case, no jump but a continuous decrease is observed. In the mixed cases, $g_{pw}(r)$ of hs-cis is closer to that in the all-trans case, and $g_{pw}(r)$ of hm-cis is more similar to that in the all-cis case. Interestingly, the amplitude of the three peaks in $g_{pw}(r)$ satisfies $g_{pw,hs-cis}(r) < g_{pw,all-trans}(r) < g_{pw,hm-cis}(r) < g_{pw,all-cis}(r)$ at $T < T_c$ (an example can be found in Figure S6). The case of hm-cis has the largest $g_{pw}(r)$, because it has the largest solvent-accessible surface area (SASA, which measures the surface area of the ELP that is accessible to solvent molecules), compared with the other cases; see Figure S7a,b. These observations are further supported by the results of the peptide–water H-bonds $N_{pw}/N_p$ shown in Figure 4c (see also Figures S8 and S9); i.e., cis isomers prevent the formation of peptide–water hydrogen bonds.

We also calculated the intramolecular H-bonds in all considered cases. Figure 4d shows the results of $N_{pp}/N_p$, the H-bonds formed between proline and the other residues of the peptide. We find that $N_{pp}/N_p$ in the all-cis case is more than twice as large as that in the all-trans case at the considered temperatures. The result of hs-cis is in between the former two cases, and that of hm-cis is the lowest. Moreover, $N_{pp}/N_p$ in the cases of hm-cis and all-cis remains essentially unchanged with temperature; it decreases in the case of hs-cis but increases monotonically in the case of all-trans as $T$ increases from $T < T_c$ to $T > T_c$. These results can be explained by the two competing effects: (i) the collapse of the chain results in more intramolecular bonds; (ii) the breaking of the $\beta$-sheets, if any, leads to fewer intramolecular H-bonds. In the cases of hm-cis and all-cis, the peptide has nearly no $\beta$-sheets, so the value of $N_{pp}/N_p$ is solely dependent on the compactness of the chain. Since the scattered trans isomers in the hm-cis case dilute the compactness effects of cis isomers, its $N_{pp}/N_p$ is even smaller than that in the all-cis case. In the hs-cis case, the number of hydrogen bonds from the block with cis isomers is barely changing as a function of $T$. In the other block with trans isomers, there are hydrogen bonds formed to stabilize the $\beta$-sheets at $T < T_c$. This set of hydrogen bonds is gone as $T > T_c$ (see Figure S4), which caused the reduction of $N_{pp}/N_p$ in the collapsed state. In the all-trans case, $N_{pp}/N_p$ slightly increases because the collapse of the chain brings more intramolecular H-bonds at $T > T_c$ (the residue separation of involved pairs along the backbone of the peptide can be seen in Figure S10). Note that there are no H-bonds formed among proline residues. Our explanation is also verified by $N_{pp}/N_p$; see Figure S10a. $N_{pp}/N_p$ increases monotonically in the cases of hm-cis and all-cis but decreases in the cases of all-trans and hs-cis as $T$ increases. Moreover, the study of a semifluid system with several chains indicates that the transition leads to strong chain overlap in the all-trans case while the all-cis chains also seem to aggregate, however, with a much weaker tendency to interpenetrate. In other words, the interactions between peptides in the all-cis case appear to be rather weak compared to those in the all-trans case; see Figure S11. Whether this is a kinetic effect or also due to the relatively short chain length studied here needs further studies.

Finally, we discuss the type of the LCST transition of (VPGVG)$_{30}$ in the all-trans case. Theoretically, both the first-order-like and second-order-like phase behavior of macro-molecules have been observed. Polyacetics are examples of the second-order-like LCST transition. Alternatively, a hysteresis between the heating and cooling procedure around the transition temperature indicates a first-order-like LCST transition. PNIPAm is one such example. In simulations, a
simple way of determining the type of phase transition is to check the distribution of the gyration radius $R_g$. A bimodal distribution of $R_g$ near the transition temperature indicates a first-order-like transition, while a unimodal distribution indicates a second-order-like transition. Figure 5 presents the $R_g$ distribution of (VPGVG)$_{30}$ at different temperatures. The bimodal distribution of $R_g$ is observed in the region $300 \, K \leq T \leq 310 \, K$, where the LCST transition occurs.

Figure 5. Distribution of $R_g$ of (VPGVG)$_{30}$ in the all-trans case at various temperatures. The bimodal distribution of $R_g$ is observed in the region $300 \, K \leq T \leq 310 \, K$, where the LCST transition occurs.

4. CONCLUSIONS

We have studied the effects of proline isomerization on the phase behavior of an ELP with sequence (VPGVG)$_{30}$ in water. Our results have shown that proline isomerization plays an important role in tuning the conformational behavior of the peptide in the LCST transition, while keeping the transition temperature $T_l$ unchanged. In particular, the peptide exhibited a expanded-to-collapsed transition if all of its proline residues were in the trans state, while no such change has been observed if all prolines were in the cis state. Moreover, we have found that the number and composition of cis proline isomers acted cooperatively in determining the global size and the propensity of secondary structure formation of the peptide. Our work may serve as an inspiration in designing new (bio)polymeric materials and opens a novel direction of regulating the phase behavior of ELPS and other proline-rich peptides.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jpcb.1c04779. Additional simulation details, supporting table, and figures (PDF)

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