Crowding effects on the structural transitions in a flexible helical homopolymer

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Abstract

We elucidate the structural transitions in a helical off-lattice homopolymer induced by crowding agents, as function of the number of monomers ($N$) and volume fraction ($\phi_c$) of crowding particles. At $\phi_c = 0$, the homopolymer undergoes transitions from a random coil to a helix, helical hairpin HH, and helix bundle HB structures depending on $N$, and temperature. Crowding induces chain compaction that can promote HH or HB formation depending on $\phi_c$. Typically, the helical content decreases which is reflected in the decrease in the transition temperatures that depend on $\phi_c$, $N$, and the size of the crowding particles.
The volume fraction ($\phi_c$) of large macromolecules such as lipids, ribosome, and cytoskeleton fibers \cite{1} in the cell interior, which can be as large as 0.4 \cite{2}, affects all biological processes ranging from transcription to folding of RNA and proteins. Protein stability \cite{3, 4} and folding rates \cite{5} of proteins are enhanced by an entropic stabilization mechanism (ESM) according to which the predominant contribution to the native state stabilization is due to an increase in the free energy of the unfolded states. Entropy decrease of the unfolded states results from the suppression of the number of allowed conformations of the polypeptide chains due to volume excluded by the crowding particles, while the native state is affected to a lesser extent. The ESM \cite{3, 5} is linked to crowding agent-induced depletion attraction \cite{5, 6, 7, 8} between the monomers of the protein or RNA \cite{9}. Crowding agents can also profoundly affect protein-protein interactions \cite{10} and amyloid formation \cite{11} that is linked to a number of neurodegenerative diseases.

We consider crowding effects on one the random coil \textbf{RC} to helix \textbf{H} transition. The interplay between the multitude of interactions between the crowding agents and proteins ($V_{CP}$) and the intra-protein forces ($V_P$) makes it difficult the structural changes that occur in a protein when $\phi_c \neq 0$. We consider the effect of spherical crowding agents on an off-lattice model of a homopolymer chain \cite{14}, which undergoes a coil to helix transition as temperature ($T$) is varied when $\phi_c = 0$. The major results, which were obtained for polymers with different $N$ and $\phi_c$ using molecular simulations, are:

(a) The phase diagram is determined by a balance between the strength, $\gamma$, of the dihedral angle potential that is related to the local stiffness and the parameter $\delta$, which specifies the strength of the hydrophobic attraction between the non-bonded beads, i.e. ones that are separated by three or more covalent bonds. As $\delta$ is varied the homopolymer undergoes a series of structural transitions from a \textbf{RC} to \textbf{H}, helical hairpin (\textbf{HH}), and helix bundle (\textbf{HB}) at low temperatures, depending on $N$. For a fixed $\phi_c$, crowding particles whose radius ($r_c$) is commensurate with the the size of the monomer ($r_m$) \cite{7, 10} ($\frac{r_m}{r_c} \approx 1$), have the largest effect in stabilizing the collapsed structures.

(b) The $r_c$ and $\phi_c$-dependent transition temperature, $T_S(r_c, \phi_c)$, from random coil (\textbf{RC}) to predominantly helical conformations (\textbf{HH} or \textbf{HB}) changes dramatically depending on $r_c$ and $\phi_c$. For a fixed $\phi_c$, the most significant change in $T_S(r_c, \phi_c)$ (compared to $\phi_c = 0$) occurs when $r_c$ is on the order of the size of the monomer. The values of $T_S(r_c, \phi_c)$ saturate, at all
\( N \), when \( r_c \) becomes large. At a fixed \( r_c \), \( T_S(r_c, \phi_c) \) decreases as \( \phi_c \) increases.

The conformations of a homopolymer chain, with \( N \) connected beads \([14]\), The chain conformations are specified by the vector \( \{ r_i \} \), \( i = 1, 2, \ldots N \). The potential energy of the chain is a sum of bond-stretch potential, bond-angle potential, interactions associated with the \((N - 3)\) dihedral angle degrees of freedom \( (V_D) \), and non-bonded potential \( (V_N) \) that determines the extent of tertiary interactions. The energy functions \( V_D \) and \( V_N \) are

\[
V_D = \sum_i \gamma \varepsilon \left\{ (1 + \cos \left( \phi_i + \frac{2 \pi}{3} \right)) + (1 + \cos 3\phi_i) \right\}, \quad \text{and} \quad V_N = \sum_{i \neq j} \varepsilon \left[ \left( \frac{2r_m}{r_{ij}} \right)^{12} - 2\delta \left( \frac{2r_m}{r_{ij}} \right)^{6} \right]
\]

where \( \varepsilon (=1 \text{kcal/mol}) \) specifies the energy of interactions between non-bonded beads \( i \) and \( j \) separated by \( r_{ij} = |r_i - r_j| \), \( \gamma (=1 \text{ in this work}) \) is the strength of the of the dihedral potential, \( \phi_i \) is the \( i^{th} \) dihedral angle, and \( r_m = 2 \text{ Å} \) is the size of a monomer. The potential \( V (= V_{CC} + V_{CP}) \), arising from interactions between the spherical crowding particles \( (V_{CC}) \) and with the monomers \( (V_{CP}) \) is

\[
V = \sum_{i \neq j} \varepsilon \left[ \left( \frac{2r_{ij}}{r_{ij}} \right)^{12} \right] + \sum_{i,j} \varepsilon \left[ \left( \frac{r_{ij} + r_{CP}}{r_{ij}} \right)^{12} \right]
\]

where \( r_{ij} \) is the distance between the crowding particles \( i \) and \( j \), \( r_{ij}^{CP} \) is the distance between bead \( i \) and the crowding particle \( j \), and \( r_c \) is size of the crowder.

The simulations were performed using a modified in-house AMBER6 \([17]\) package that was altered to incorporate Langevin dynamics in the low friction limit \([18]\) to enhance the rate of conformational sampling \([19]\). In the presence of crowders the calculations were performed in the NVT ensemble. The homopolymer and the crowding particles are confined to a cubic box and periodic boundary conditions are used to minimize surface effects. The size of the simulation box, is determined by the condition that the box contain a minimum of 150 crowding particles. For small \( r_c \), the edge of the box is equal to the sum of the length of the fully extended helix and four times the average distance between the crowders at a specified \( \phi_c \). The number of crowding particles ranges from \( \approx 150 \) for the most dilute system with the largest \( r_c \), to 1200 \( (\phi_c = 0.2, r_c = 2 \text{ Å}, N = 16) \), and 3000 \( (\phi_c = 0.2, r_c = 4 \text{ Å}, N = 64) \).

The sampling efficiency in the simulations are enhanced using the replica exchange method (REM) \([3, 15]\), which ensures that the thermodynamic averages are fully converged. We used twenty replicas in the temperature range from \( T=100 \) to 400K in the REM simulations. The initial configurations are randomly chosen from high-temperature simulations, and subsequently quenched to the desired temperatures. The integration time step is \( 10^{-4}\tau_L \) where \( \tau_L = \left( \frac{m r_m^2}{\varepsilon} \right)^{\frac{3}{2}} \) with \( m \) being the mass of a bead. At chosen time interval \( (= 40\tau_L) \), config-
urations with neighboring temperatures are exchanged. The acceptance probability, which depends on the temperatures and the energies of the replicas, was in the range 0.2–0.3. In order to calculate averages we retained between (4,000 - 8,000) conformations for each replica. The results of the REM simulations were combined with independent data set generated using the weighted histogram analysis method (WHAM) to obtain thermodynamic averages. The structural transitions in the homopolymer are characterized by using the specific heat $C_V$ and the radius of gyration $R_g$. The extent of helical order was quantified using $f_H(T, \phi_c) = \frac{1}{N-3} \sum_{i=1}^{N-3} \langle \Theta(\Delta \phi - |\phi_i - \phi_i^N|) \rangle$ where $\Theta(x)$ is the Heaviside function, $\phi_i^N$ is the value of the $i^{th}$ dihedral angle in the energy minimized ($T = 0$) helical state, and $\Delta \phi$ is the tolerance in $\phi_i$ used to assign helical character to the $i^{th}$ dihedral angle. We chose $\Delta \phi = 12.07^\circ$ to ensure that $T_S$ obtained using the criterion $f_H(T_S, \phi_c = 0) = 0.5$ is consistent with the temperature at which $C_V$ for $N = 64$ has a maximum.

The structural transitions as a function of $T$ and $\delta$ for $N = 16$ with $\phi_c = 0$ (Fig. 1a) show that the $\text{RC} \rightarrow \text{H}$ transition occurs at $T_S \approx 292$ K at $\delta = 0$. For low to moderate $\delta$ values ($\delta \lesssim 0.5$) the polymer exists either as a $\text{RC}$ or a $\text{H}$. With $\delta = 0.75$, we find a transition to a $\text{HH}$ that is accompanied by a drastic reduction in $R_g$. At high $\delta$ values, the energy cost to form a bend in $\text{HH}$ is compensated by a number of favorable tertiary contacts that stabilize the $\text{HH}$. The chain compaction at high $\delta$ results in structures that have high helical content as measured. For $N = 32$ and 64, besides $\text{H}$ and $\text{HHs}$ we find that helix bundles ($\text{HBs}$) can also form as $\delta$ and $T$ are changed. For some range of $\delta$ the ordered structures coexist, while for other choices the probability distribution is peaked around only one unique structure. The transition temperature $T_S$ at which the ordered structures form changes dramatically as $\delta$ increases (Fig. 1b). Only when $\delta > 0.5$, do we find significant dependence of $T_S(\delta, \phi_c)$ on $N$ (Fig. 1b).

From the temperature dependence of the thermally averaged $R_g$ with $\phi_c = 0.2$ and at various sizes of $r_c$ we find that smaller crowding agents ($r_c = 2$ or 4 Å) are most efficient in inducing chain compaction (Fig. 2a). For $r_c = 2$ Å the values of $R_g$ even at high temperatures are considerably smaller than $R_g^H$ - the radius of gyration of the energy-minimized helical structure ($T = 0$). A qualitative explanation follows from the Asakura-Oosawa (AO) theory, which predicts that the strength of the additional entropically-induced effective attraction between the beads increases as $r_c$ decreases and $\phi_c$ increases. As a result, the
effective attraction $\delta_R(\phi_c, r_c) \sim \delta_0 + f(\phi_c, r_c)$, which increases with decreasing $r_c$, is largest for small $r_c$. In the helical homopolymer model a reduction in $R_g$, at sufficiently large $\delta$, is also accompanied by enhancement in helical order which explains the emergence of HH (Fig. 2b). Thus, crowding agents with $\frac{r_m}{r_c} \approx 1$ are most efficient in inducing ordered structure formation at high $T$ even if $\delta$ is not large.

The probability density, $P(T, R_g/R_H)$, in Fig. 2b for $\phi_c = 0.2$, shows that the distribution function changes continuously as $T$ decreases. At high temperatures, there is one broad peak that represents an ensemble of mostly random coil RC structures. As $T$ decreases below $T_S(r_c, \phi_c)$ a sharp peak that corresponds to HH structures, with high $f_H(T, \phi_c)$, emerges. Since there is only a continuous shift in $P(T, R_g/R_H)$, without a discernible region of bimodality, the transitions to structures with high $f_H(T, \phi_c)$ are not ”phase transitions”. Rather, the energy landscape has multiple basins of attraction with varying helical content whose population can be altered by changing $T$, $\phi_c$, or $\delta$.

Transitions to higher order (three or more) HB structures occur for $N = 64$ with $\phi_c = 0.2$ and $r_c = 4$ Å (Fig. 3). In this case, crowding-induced formation of HH and HB at low $T$ is also accompanied by a dramatic reduction in $R_g$ (Fig. 3a). As temperature decreases, there is evidence for coexistence between long HH and HB (Fig. 3b). The formation of a large number of inter-helical contacts compensates for the energetic cost due to bend formation which results in the transition to the HB.

The volume fraction $\phi_c$ can be independently altered by changing either $r_c$ or the number density of the crowding agents. For a fixed $N$ and $r_c$, the values of $T_S(r_c, \phi_c)$ decrease as $\phi_c$ increases (Fig. 4a). The variations are larger for the smaller $r_c$ (Fig. 4a). The decrease in $T_S(r_c, \phi_c)$ with increasing $\phi_c$ is a consequence of the enhancement in $\delta_R(\phi_c, r_c)$ caused by the entropic depletion attraction. From the AO theory, it follows that the strength of the depletion attraction $f(\phi_c, r_c)$ should increase as $\phi_c$ increases. Thus, $T_S(r_c, \phi_c)$ should have the largest shift as $\phi_c$ increases, which is in accord with our simulations (Fig. 4a).

The transition temperatures $T_S(r_c, \phi_c)$ (obtained using $f_H(T_S, \phi_c) = 0.5$) reports on the total helical content independent of whether the structure is a H, HH or HB. The changes in $T_S(r_c, \phi_c)$ for a fixed $\phi_c$ and varying $r_c$ are shown in Figure 4A. We expect that as $r_c$ increases beyond $R_g^H$ the transition temperature $T_S(r_c, \phi_c)$ should approach the value expected for folding in narrow confined space formed by large crowding agents, and hence be
independent of $r_c$. This is borne out by the simulations which show that $T_S(\phi_c, r_c)$ is almost constant as $r_c > 30$ Å (Fig. 4b). For smaller values of $r_c$ Fig. 4b shows that $T_S$ decreases sharply especially for $N = 16$. As in Fig. 4a, we find that the largest changes are obtained for $r_c = 2$ Å.

The stability of helical conformations is determined by interplay of the local stiffness and the specific attractive interactions between beads $i$ and $i + 3$. In contrast, crowding agents, which enhance non-specific homogeneous attraction between the beads, induce chain compaction. Whether chain compaction is also accompanied by enhanced helical stability depends on the range and the strength of the AO attraction. At all values of $r_c$, the transition temperatures $T_S(r_c, \phi_c)$ decrease as $r_c$ decreases with the change being most dramatic for small $r_c$ (Fig. 4b). These results show that the helical stability decreases when $\phi_c$ is non-zero even though the chain is compact. Thus, we conclude that the homogeneous AO attraction compromises the forces required to stabilize particular (H, HH, or HB) helical states. If the polymer backbone were stiff ($\gamma > 1$) then stretches of helical conformation on the scale of the persistence length of the chain would persist especially at low temperatures [8]. For the flexible helical polymer there is a loss in helical stability, which is manifested by a decrease in $T_S(r_c, \phi_c)$ that is most pronounced when $r_c$ is small.

We conclude with the following remarks. (1) Variation of the non-zero hydrophobicity parameter $\delta$ leads to a variety of higher order structures at low temperatures, such as HH and HB, whose stabilities can be enhanced by macromolecular crowding. (2) The high temperature denatured structures are more compact at $\phi_c \neq 0$ (see Figs. 2a and 3a) than when $\phi_c = 0$, which has implications for crowding-induced folding mechanisms of proteins. (3) The prediction that the helix stability changes, as $\phi_c$ and $r_c$ are varied, can be validated using circular dichroism (CD) spectroscopy that detects the extent of helix formation in proteins [22].

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Figure Captions

Figure 1. (a) Normalized radius of gyration $R_g/R^H_g$ as a function of temperature for several values of the short-range attraction parameter $\delta$ for the $N = 16$ chain. Snapshots of typical conformations are also shown. The value of $R^H_g = 10.1 \text{ Å}$. (b) Transition temperature $T_S$ as a function of $\delta$ for $N = 16, 32$ and 64.

Figure 2. (a) Normalized radius of gyration $R_g/R^H_g$ as a function of $T$ for different crowder radii $r_c$ (indicated in the Figure) at $\phi_c = 0.2$ and $N = 16$. (b) Probability distribution functions of $R_g/R^H_g$ for different temperatures for $\phi_c = 0.2$ and $r_c = 2 \text{ Å}$ case in (a).

Figure 3. Same as Fig.2 except $N=64$. (a) Normalized radius of gyration $R_g/R^H_g$ (with $R^H_g = 40.4 \text{ Å}$) at constant $\phi_c = 0.2$. (b) Probability distribution functions of $R_g/R^H_g$ for the $\phi_c = 0.2$ and $r_c = 4 \text{ Å}$ case shown in (a).

Figure 4. (a) Helix-coil transition temperature $T_S(r_c, \phi_c)$ as a function of $\phi_c$ at a constant $r_c$ for three chains with $N = 16$ ($r_c = 2 \text{ Å}$), $N = 32$ ($r_c = 4 \text{ Å}$), and $N = 64$ ($r_c = 4 \text{ Å}$). (b) $T_S(r_c, \phi_c)$ as a function of the crowder radius $r_c$ at $\phi_c = 0.2$. Plots for chains of lengths $N = 16, 32$ and 64 are shown. In both (a) and (b) $\delta = 0.3$.
\( \phi = 0.2 \)

\( r_c = 2A \)

\( N = 16 \)

\( \phi_c = 0.2 \)

\( \delta = 0.5 \)

\( r_c = 32Å \)

\( r_c = 16Å \)

\( r_c = 8Å \)

\( r_c = 4Å \)

\( r_c = 2 \)

(b) 

Probability density

\( R_g/R_g^H \)

\( T, K \)

\( R_y/R_y^H \)
(a)\[
\frac{R_g}{R_g^H} = \begin{cases}
0 & \text{no crowding} \\
0.0 & r_c = 32 \, \text{Å} \\
0.1 & r_c = 16 \, \text{Å} \\
0.2 & r_c = 8 \, \text{Å} \\
0.3 & r_c = 4 \, \text{Å}
\end{cases}
\]

(b)\[
\begin{array}{c}
\text{N=64} \\
\phi_c = 0.2 \\
\delta = 0.3
\end{array}
\]

\[
T, K
\]

\[
0.0  \quad 0.1  \quad 0.2  \quad 0.3  \quad 0.4  \quad 0.5  \quad 0.6  \quad 0.7  \quad 0.8  \quad 0.9  \quad 1
\]

\[
\frac{R_g}{R_g^H}
\]

\[
\text{Probability density}
\]

\[
T.K
\]

\[
0.0  \quad 0.2  \quad 0.4  \quad 0.6  \quad 0.8  \quad 1
\]

\[
R_g/R_g^H
\]
S

\(N=16, r_c = 2 \text{ Å}\)
\(N=32, r_c = 4 \text{ Å}\)
\(N=64, r_c = 4 \text{ Å}\)

(a)

(b)