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Helix formation inside a nanotube: Possible influence of backbone-water hydrogen bonding by the confining surface through modulation of water activity

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Recent molecular dynamics simulations of Sorin and Pande [J. Am. Chem. Soc. **128**, 6316 (2006)] in explicit solvent found that helix formation of an alanine peptide is disfavored inside a nanotube relative to that in bulk solution. Here, we present a theory to quantitatively rationalize their simulation results. The basic idea is that the nonpolar inner surface of the nanotube creates a depletion layer and raises the activity of the confined water. The raised water activity, in turn, stabilizes the coil state through hydrogen bonding with the backbone amides and carbonyls. We account for the influence of water activity on helix formation within the Lifson-Roig theory. With physically reasonable parameters, the dependence of the helical content on the diameter of the nanotube obtained in the simulations is well reproduced. © *2007 American Institute of Physics*. [DOI: 10.1063/1.2812282]

I. INTRODUCTION

Confinement may significantly affect the enthalpic and entropic factors important for protein folding. The central cavity of chaperonins[1] and the exit tunnel of the ribosome[2] illustrate the potential importance of confinement in *in vivo* protein folding. Different aspects of confinement have been studied by theoretical modeling,[3–7] molecular dynamics (MD) simulations,[5,8–11] and experiments[1,2,12–16] (for a recent review, see Ref. 17). Two recent MD simulation studies by Pande and co-workers[18,19] are notable in two respects. Unlike all previous modeling and simulations, they included water explicitly in their simulations, and contrary to previous studies they found confinement to have a destabilizing effect. To rationalize the decreased helix stability of an alanine peptide inside a nanotube observed in their MD simulations, Sorin and Pande[18] argued that water released upon peptide backbone-backbone hydrogen bonding has less translational entropy inside the nanotube than in bulk solution and the reduced gain in translational entropy upon helix formation is the cause for the destabilization. However, their theoretical analysis along this line of argument seems to contain errors. Here, we propose an explanation based on the idea that the inner surface of the nanotube, by its nonpolar nature, increases the activity of the confined water. The increased water activity favors the coil state since the backbone amides and carbonyls are then free to make hydrogen bonds with the water molecules. We carry out a quantitative analysis and show that the simulation results of Sorin and Pande can be reproduced with physically reasonable parameters.

In Sec. II, we outline the theoretical procedure to account for the effect of modulated water activity on helix formation. Section III presents the main results of our analysis and our conclusion. Details of our analysis are given in Appendix A; comments on possible errors in Ref. 18 are presented in Appendix B.

II. THEORY

Our analysis is based on the helix-coil transition theory of Lisfon and Roig.[20] Modified versions of the theory have been developed,[21] but here we use the original theory for simplicity. In this theory, a residue in the coil state is assigned a statistical weight of 1. In the helix state, there are two possibilities: the backbone of the residue is still free to make hydrogen bonds with water or is already hydrogen bonded to backbone groups of the neighboring residues. The statistical weights assigned to the two possibilities are v and w (modeling helix initiation and propagation), respectively. For future references, the coil state, the helix state free of backbone-backbone hydrogen bonding, and the helix state with backbone-backbone hydrogen bonding are denoted as c, h_f, and h_h, respectively. The partition function of a peptide with N residues is written as $Z(v,w;N)$. The average number of hydrogen-bonded helical residues is given by

$$N_{\text{helix}} = \frac{\partial \ln Z(v,w;N)}{\partial \ln w}. \tag{1}$$

Implementation of the Lifson-Roig theory is presented in Appendix A.

In the Lifson-Roig theory water activity is accounted for only implicitly. We now explicitly consider the effect of water activity by adapting the work of Bixon and Lifson.[22] According to that work, a solvent that can hydrogen bond to the peptide backbone amides and carbonyls contributes a factor

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$$z(a) = (1 + K_{\mathrm{NH}}a)(1 + K_{\mathrm{CO}}a) \tag{2}$$

to the statistical weight of each residue when it is available for hydrogen bonding with the solvent. In this expression, K_{NH} and K_{CO} are the equilibrium constants for hydrogen bond formation with the solvent by the backbone amide and carbonyl, respectively, and a is the activity of the solvent. The solvent for our purpose is water.

Of the three states of each residue, the backbone is free to hydrogen bond with water only in c and h_f. Hence, their statistical weights, 1 and v, respectively, should be multiplied by $z(a)$. This is equivalent to keeping the weights of these two states at 1 and v but dividing by $z(a)$ the weight w of the third state (i.e., h_h). If the helix initiation and propagation parameters appropriate for bulk water are v and w, then their counterparts are v and $wz(a)/z(a^c) \equiv w^c$ when water activity is changed from the bulk water a to a^c. At the new water activity, the average number of hydrogen-bonded helical residues is given by

$$N_{\mathrm{helix}}{}^{c} = \frac{\partial \ln Z(v, w^c; N)}{\partial \ln w^c}. \tag{3}$$

To proceed further, we now make a number of simplifying assumptions. The first is to assume $K_{\mathrm{CO}}a$ and $K_{\mathrm{NH}}a \gg 1$, which amounts to the assumption that the backbone amides and carbonyls in the c and h_f states are always hydrogen bonded to water. This leads to

$$w^c = w(a/a^c)^2. \tag{4}$$

$N_{\mathrm{helix}}{}^{c}$ monotonically increases with increasing w^c; hence an increase in water activity leads to a decrease in w^c and, consequently, a lower helical content.

Next we assume that the nonpolar surface of the nanotube increases the activity of the confined water through creating a depletion layer.[23] Furthermore, we approximate the ratio of activities by the ratio of concentrations. Let the diameter and length of the nanotube be d and L, respectively.[24] If the volume of the peptide is V_p, then the number of water molecules in the free volume, $\pi d^2L/4 - V_p$, will be $a(\pi d^2L/4 - V_p)$ if the water concentration is the bulk activity a. Now these water molecules are partitioned into two regions. In the depletion layer (with thickness δ) next to the inner surface of the nanotube, let the water activity be a^s. In the rest of the free space, the water activity is a^c, which already appeared in Eq. (3) through w^c. We have

$$a(\pi d^2L/4 - V_p) = a^s[\pi d^2L/4 - \pi(d-2\delta)^2L/4] + a^c[\pi(d-2\delta)^2L/4 - V_p]. \tag{5}$$

Rearrangement leads to

$$\frac{a^c}{a} = \frac{\pi d^2L/4 - V_p - \pi\delta(d-\delta)La^s/a}{\pi(d-2\delta)^2L/4 - V_p}. \tag{6}$$

III. RESULTS AND DISCUSSION

Equations (3), (4), and (6) give prediction for the dependence of the helical content on the nanotube diameter, which can be compared against the simulation results of Sorin and Pande.[18] Most of the parameters involved are either fixed by the simulations (e.g., $N=23$ and $L=89.3$ Å) or have very little room for adjustment (e.g., $v=0.036$ and $w\sim1.5$ according to Rohl *et al.*;[21] δ is probably about one layer of water or ~3 Å; the lower limit of V_p is the volume of the all-helix peptide, which is ~75 Å^3 per residue). In Fig. 1, we compare the predicted $N_{\mathrm{helix}}{}^{c}$ with the simulation results. The agreement seems very good. The values of the parameters used are $w=1.45$, $\delta=3.2$ Å, $V_p=100$ Å^3 per residue, and $a^s/a=0.969$.

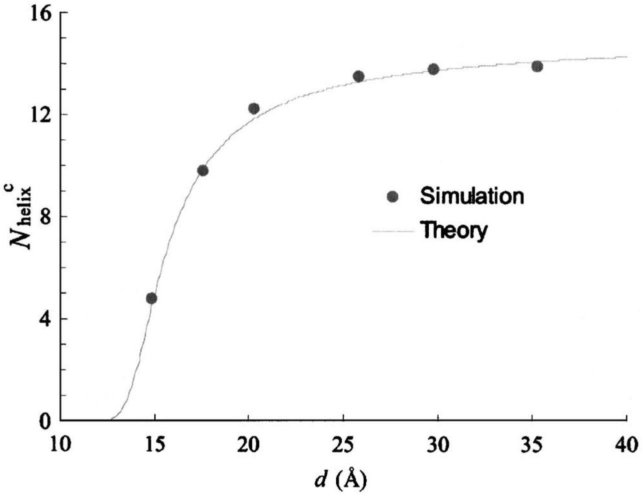

FIG. 1. Comparison of the predicted dependence of the helical content on the diameter of the confining nanotube against the simulation results of Sorin and Pande (Ref. 18).

The theory also predicts the average number of backbone-water hydrogen bonds.[22] This is given by

$$\langle \#\mathrm{HB}_{\mathrm{pw}} \rangle = (N - N_{\mathrm{helix}}{}^{c})[K_{\mathrm{NH}}a^c(1+K_{\mathrm{NH}}a^c)^{-1} + K_{\mathrm{CO}}a^c(1+K_{\mathrm{CO}}a^c)^{-1}]/2. \tag{7}$$

When $K_{\mathrm{CO}}a^c$ and $K_{\mathrm{NH}}a^c \gg 1$, the right-hand side of Eq. (7) approaches $N - N_{\mathrm{helix}}{}^{c}$. That limiting value corresponds to a linear relation between $\langle \#\mathrm{HB}_{\mathrm{pw}} \rangle$ and $N_{\mathrm{helix}}{}^{c}$, with a slope of -1. Sorin and Pande found a strong correlation between $\langle \#\mathrm{HB}_{\mathrm{pw}} \rangle$ and $N_{\mathrm{helix}}{}^{c}$ but with a slope of -0.69. That slope can be obtained from Eq. (7), e.g., when $K_{\mathrm{CO}}a^c \sim K_{\mathrm{NH}}a^c \sim 2.2$.

The simulation studies of Pande and co-workers with explicit solvent raise important questions about the interplay between the confining surface, the confined water, and the confined protein molecule. Here, we have focused on the influence of backbone-water hydrogen bonding by the confining surface through modulating water activity. The stability of a confined protein molecule may also be affected by a number of other factors, such as the concurrent reductions in statistical weights of the folded and unfolded states; the former is due to restriction in translational and rotational placement of the protein inside the confining cage and the latter is due to the elimination of conformations crossing the surface of the cage.[3–7] A shown by MD simulations,[10,11] attractive interactions (e.g., arising from hydrophobic effects) between the confined protein and the confining surface can significantly influence the folding equilibrium and kinetics. With regard to the role of the translational entropy of water released upon protein structure formation, the theory presented here actually takes that into consideration through the equilibrium constants K_{NH} and K_{CO} and the water activity (that is, the differences of these quantities between a confined environment and bulk solution may arise in part from the restricted translation of the confined water). However, it is our belief that the fundamental reason for the helix destabilization observed in the simulations of Pande and coworkers is the nonpolar surface of the confining cage.[25] As Mukherjee *et al.* found experimentally,[16] helix stability is increased inside AOT reverse micelles, which have charged inner surfaces. The increased stability is expected from the theory presented here;[23] it will be interesting to see to what extent quantitative rationalization of those experimental results can be achieved.

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APPENDIX A: IMPLEMENTATION OF THE LIFSON-ROIG THEORY

Here, we present the implementation of the Lifson-Roig theory[20] for helix-coil transition. Each residue of a peptide can be in three distinct states: c, the coil state; h_f, a helix state in which the backbone is free to hydrogen bond with water; and h_h, a helix state in which the backbone is hydrogen bonded to neighboring backbone groups. The statistical weights of the three states are 1, v, and w_c, respectively. A peptide with N residues can sample 3^N distinct conformations. The total partition function is given by

$$Z=(0\ 0\ 1)W^N\begin{pmatrix}0\\1\\1\end{pmatrix}, \tag{A1}$$

where the weight matrix is given by

$$W=\begin{bmatrix}w^c & v & 0\\ 0 & 0 & 1\\ v & v & 1\end{bmatrix}. \tag{A2}$$

The eigenvalues of the weight matrix are the three roots of the cubic equation

$$(\lambda-w^c)(\lambda^2-\lambda-v)-v^2=0. \tag{A3}$$

For future reference, the three eigenvalues are denoted as λ_j, $j=1$, 2, and 3. In terms of the three eigenvalues, the partition function is given by

$$Z=\sum_{j=1}^{3}Z_j, \quad Z_j=\lambda_j{}^N C_j, \tag{A4a}$$

where

$$C_j=\lambda_j{}^2 v^2/[\mu_j{}^2+(2\lambda_j-1)v^2], \quad \mu_j=\lambda_j{}^2-\lambda_j-v. \tag{A4b}$$

The average number of hydrogen-bonded helical residues, given by Eq. (3) of the main text, can now be written as

$$N_{\mathrm{helix}}{}^c=\sum_{j=1}^{3}N_jZ_j/Z, \quad N_j=n_{j0}(N+n_{j1}), \tag{A5a}$$

where

$$n_{j0}=\frac{\mu_j}{\lambda_j}\frac{\lambda_j\mu_j-v^2}{\mu_j{}^2+(2\lambda_j-1)v^2},$$

$$n_{j1}=2\left[1-\lambda_j\frac{(2\lambda_j-1)\mu_j+v^2}{\mu_j{}^2+(2\lambda_j-1)v^2}\right]. \tag{A5b}$$

To find the dependence of the helical content on the diameter of the nanotube, we first calculate a^c/a according to Eq. (6) and then find w^c according to Eq. (4) (all equation numbers refer to those in the main text). For this value of w^c, the helical content is finally found through Eqs. (A5a) and (A5b).

All parameters, except for a^s/a and w, are assigned the values listed in the main text. The values of a^s/a and w are fitted. Specifically, the value of w^c giving rise to the simulation result for $N_{\mathrm{helix}}{}^c$ at each nanotube diameter d is obtained. The dependence of w^c on d is then fitted to the function obtained from combining Eqs. (4) and (6), with a^s/a and w as floating parameters. The best-fit values of a^s/a and w are reported in the main text and are used to generate the theory curve in Fig. 1.

APPENDIX B: COMMENT ON THE THEORY OF REFERENCE 18

In the main text we point out that the theoretical analysis of Sorin and Pande[18] seems to contain errors. Here we elaborate on this statement.

Sorin and Pande presented the theory in Supporting Information, which is referred to here as SP-SI. The final result of their theory is given in section (e) of SP-SI. Their starting equation, Eq. (1), had the sign of the free energy $\Delta G(d)$, wrong. This error was inherited from section (d) of SP-SI, where a model for hydrogen bonding of water proposed by Nadler and Krausche[26] was adopted. Their expression for $\Delta G(d)$, the free energy for peptide-petide hydrogen bonding in a nanotube with diameter d, was given in Eq. (3). In this expression, the sign of the entropy term (i.e., the second term) also appears to be wrong. Sorin and Pande had $\Delta S(d)=-k_B\ln[\pi(d_{\mathrm{eff}}/2)^2/A_{H_2O}]$, which is explicitly given in the main text and would predict that entropy decreases with increasing d_{eff}. However, physically one expects entropy to increase with increasing d_{eff}.

Beyond sign errors, one can also question whether the equations in section (e) of SP-SI make physical sense. For example, one expects the free energy $\Delta G(d)$ to approach the bulk value ε_{HB} as $d\to\infty$. Equation (3) in section (e) of SP-SI, with or without sign correction, stands in contradiction to this expectation.

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[23] Conversely, one can argue that a confining cage with a charged inner surface, such as formed by a sodium bis(2-ethylhexyl) sufosuccinate (AOT) reverse micelle, will create an "enrichment" layer and hence lead to a reduced water activity. That, in turn, will be predicted to increase helix stability. Indeed, Mukherjee *et al.* (Ref. 16) observed experimentally helix stabilization inside AOT reverse micelles and proposed an argument along this line to rationalize the stabilization.
[24] In the simulations of Sorin and Pande (Ref. 18), the system is periodic along the axis of the nanotube. In that case L is the nanotube length for each copy of the peptide.
[25] In another study, Lucent *et al.* (Ref. 19) simulated the folding of the villin headpiece inside a spherical cavity. They found that the folding stability is increased when the cavity is made permeable to water, in agreement with the theoretical prediction of the confinement (Ref. 3), but is decreased when water is totally confined in the cavity. These results are qualitatively consistent with the theoretical analysis presented here. When the cavity is permeable, water activity is not perturbed. On the other hand, when water is fully confined, its activity will be increased by the nonpolar wall of the cavity. Again, the increased water activity favors the unfolded state by allowing for backbone-water hydrogen bonds. We argue that the destabilizing effect of the increased water activity dominates over the stabilizing effect of confinement.
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