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SHORT COMMUNICATION

Effect of mixed macromolecular crowding agents on protein folding

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ABSTRACT

In cells, proteins fold and unfold in the presence of macromolecules with various sizes and shapes. Recent experiments by Liang and coworkers (J Biol Chem 2004;279:55109–55116; J Mol Biol 2006;364:469–482) show that protein refolding is enhanced by a mixture of two different crowding agents relative to the individual crowding agents and an optimal mixing ratio exists. Here, we present a theory that predicts the existence of an optimal mixing ratio. The theory is based on models for calculating the changes in the chemical potentials of the folded and unfolded states by a mixture of crowders. The existence of an optimal mixing ratio results from the dependences of these chemical-potential changes on crowder sizes and concentrations, which can be argued to be quite general. We further predict that, for any crowding agent, the stabilizing effect can be optimized both by varying the molecular weight and the mixing ratio of two species with different molecular weights.

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Key words: excluded-volume effect; folding stability; folding rate.

INTRODUCTION

Most of our knowledge on protein folding has been gained through studies in dilute solutions. To understand protein folding under physiological conditions, in a growing number of studies, some aspects of the complex cellular environments have been mimicked.[1] In particular, Liang and coworkers[2,3] studied the effect of a mixture of two crowding agents and found that the refolding rates and yields can be optimized by varying the mixing ratio of the crowding agents. Here, we present theoretical models for the effects of a mixture of crowding agents on the folding state, a compact transition state, and the unfolded state. These models together predict an optimal mixing ratio for folding stability and folding rate.

Our models build on earlier theoretical studies.[4–8] In addition, atomistic simulations[9–11] have provided important insight into the interactions of solute proteins with crowding agents. We show that the existence of an optimal mixing ratio results from general relations of crowding effects to crowder sizes and concentrations. New predictions on crowding effects are also made.

THEORY

The effect of crowding on a biochemical process is related to the differential effects of crowding on the end states.[1] In the case of the folding equilibrium of a protein, according to the thermodynamic cycle illustrated in Figure 1, the change in the folding free energy by crowding, $\Delta\Delta G_f = \Delta G_f - \Delta G_{f0}$, can be calculated as

$$\Delta\Delta G_f = \Delta\mu_F - \Delta\mu_U \quad (1)$$

where $\Delta\mu_X$ is the change in the chemical potential of state X by crowding. Here, X is either the folded protein ("F") or unfolded protein ("U"). The same procedure can also be used to obtain the change, due to crowding, in the activation free energy for folding:

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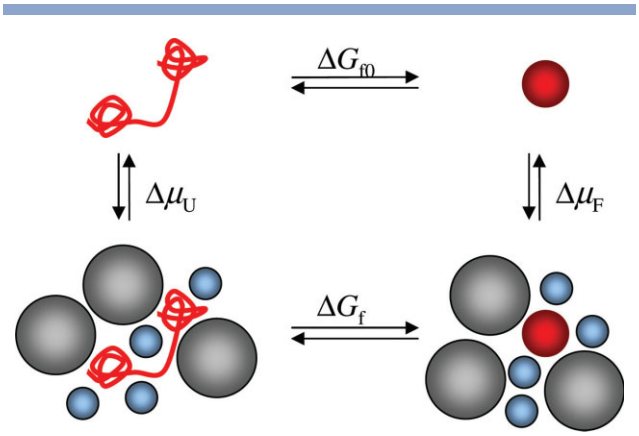

Figure 1

Thermodynamic cycle for obtaining the effect of crowding on the folding stability. ΔG_{f0} and ΔG_f, respectively, are the folding free energies in the absence and presence of crowding. $\Delta\mu_U$ and $\Delta\mu_F$ are the changes in the chemical potentials of the unfolded and folded states, respectively, due to crowding.

$$\Delta\Delta G^{\ddagger} = \Delta\mu_{TS} - \Delta\mu_U \quad (2)$$

where the subscript TS refers to the transition state for folding.

We model a mixture of macromolecular crowders as hard spheres with different radii. Let the radius and concentration of species i be R_i and c_i, respectively. We now examine how a mixture of crowders affects the chemical potentials of the folded and unfolded states.

Folded state

We model a folded protein as a sphere, with a radius R_F. $\Delta\mu_F$ is the same as the work of inserting a single-folded protein molecule at some fixed position inside the mixture of crowders. According to the scaled particle theory,[12] one has

$$\begin{aligned}\Delta\mu_F/k_BT = &-\ln(1-\phi) + (1-\phi)^{-1} \\ &\times \sum_i \phi_i(3z_i + 3z_i^2 + z_i^3) \\ &+ (1-\phi)^{-2}\sum_i \phi_i z_i \sum_i \phi_i(9z_i/2 + 3z_i^2) \\ &+ 3(1-\phi)^{-3}\left(\sum_i \phi_i z_i\right)^3 \end{aligned} \quad (3)$$

where k_BT is thermal energy, $z_i = R_F/R_i$,

$$\phi_i = (4\pi/3)R_i^3 c_i \quad (4)$$

is the volume fraction of species i crowder, and $\phi = \sum_i \phi_i$. Note that, if the transition state for folding is also modeled as a sphere (somewhat enlarged relative to the sphere modeling the folded state), as is done in previous studies,[7] then its change in chemical potential by crowding is given by Eq. (3) as well, except that R_F is replaced by an enlarged R_{TS}.

In Eq. (4), the crowder concentration is expressed as number density. If the crowder concentration is in grams per liter, the volume fraction is given by $\phi_i = 2.52 \times 10^{-3}(R_i^3/M_i)c_i$, where M_i is the molecular weight of the crowder. If the molecular weight is assumed to be proportional to the volume of a crowder molecule, then ϕ_i is proportional to c_i in grams per liter. For a protein-like molecule, $\phi_i \approx 1.2 \times 10^{-3}c_i$, and a crowder concentration of 100 g/L corresponds to a volume fraction of 0.12.

When there is just a single species of crowder, Eq. (3) reduces to

$$\begin{aligned}\Delta\mu_F/k_BT = &-\ln(1-\phi) + (1-\phi)^{-1}\phi(3z_1 + 3z_1^2 + z_1^3) \\ &+ (1-\phi)^{-2}\phi^2(9z_1^2/2 + 3z_1^3) + 3(1-\phi)^{-3}\phi^3 z_1^3 \end{aligned} \quad (5)$$

Unfolded state

We model an unfolded protein as a Gaussian chain. Such a model was first used in analyzing the effect of confinement on the equilibrium of protein folding;[5] the resulting theory has found wide applications [see Refs. 1 and 8 for recent reviews]. When the crowders are present, only a fraction of all possible conformations of the Gaussian chain are free of intersections with the crowders. Let this fraction be $S(N)$, where N is the number of residues in the protein. The change in the chemical potential of the unfolded protein is then given by[6,8]

$$\Delta\mu_U/k_BT = -\ln(1-\phi) - \ln S(N) \quad (6)$$

A Gaussian chain is equivalent to the trajectory of a Brownian particle with an effective diffusion constant $D_{eff} = b^2/6$ (b: root-mean-square-displacement of two adjacent residues), and the fraction $S(N)$ is identical to the survival probability of the Brownian particle when the crowders act as static traps.[6] In the mapping from the Gaussian chain to the Brownian particle, N plays the role of time. The survival probability is given by[13,14]

$$\begin{aligned}-\ln S(N) = &\ 4\pi R_g^2 \sum_i c_i R_i(1 + 2R_i/\pi^{1/2}R_g) \\ &- (4\pi R_g)^2 \sum_{i,j} c_i c_j R_i^2 R_j^2 K_{i,j} \end{aligned} \quad (7)$$

where $R_g = (Nb^2/6)^{1/2}$ is the radius of gyration of the unfolded protein. The coefficients K_{ij} are positive. When $R_g \geq R_i$ for each species of crowder, Berezhkovskii derived the following approximate result:

$$K_{ij} = \ln(R_g/R_{i,j>}) \quad (8)$$

where $R_{i,\,j>}$ is the larger of R_i and R_j. Terms higher than second order in crowder concentration have been

neglected in Eq. (7), which make a positive contribution to $-\ln S(N)$ and are probably negligible when the first term of Eq. (7) is $<\sim 7$.[13]

Combining the last three equations, we have

$$\Delta\mu_U/k_BT = -\ln(1-\phi) + 3\sum_i \phi_i y_i^2(1 + 2/\pi^{1/2} y_i) - 9\sum_{i,j} \phi_i\phi_j y_i y_j \ln y_{i,j>} \quad (9)$$

where $y_i = R_g/R_i$ and $y_{i,\,j>} = R_g/R_{i,\,j>}$. In the presence of a single species of crowder, Eq. (9) reduces to

$$\Delta\mu_U/k_BT = -\ln(1-\phi) + 3\phi y_1^2(1 + 2/\pi^{1/2} y_1) - 9\phi^2 y_1^2 \ln y_1 \quad (10)$$

Note that both $\Delta\mu_F$ and $\Delta\mu_U$ contain the term $-\ln(1-\phi)$, which can be removed without any consequence on the folding or activation free energy.

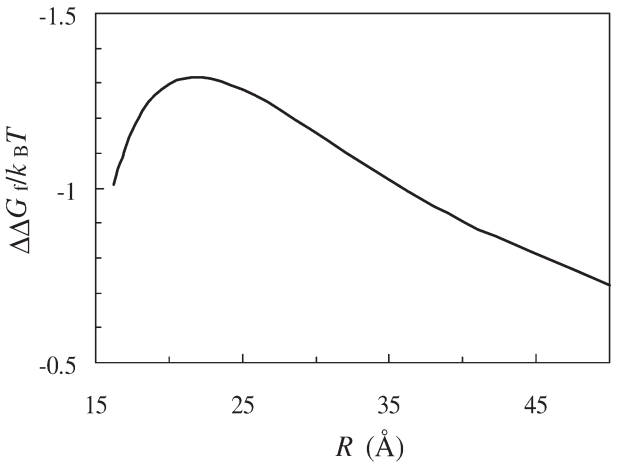

Figure 2

Variation of the crowding effect on folding stability with the crowder size. A single species of crowder, at a concentration of 150 g/L, is present.

RESULTS AND DISCUSSION

We illustrate the effect of crowding on a protein modeled as a sphere with a radius $R_F = 20$ Å in the folded state and as a Gaussian chain with a radius of gyration $R_g = 50$ Å in the unfolded state (roughly corresponding to a protein with 200 residues). For comparison, we also present some results for a protein with 100 residues, but unless otherwise specifically indicated, results are for the 200-residue protein.

Optimal size for a single species of crowder

Equations (5) and (10) give the changes in the chemical potentials of the folded and unfolded states, respectively, due to the presence of a single species of crowder. Both expressions are nonlinear in the radius, R_1, of the crowder. There is thus a possibility that an optimal crowder radius exists, at which the stabilization effect of crowding is maximal. In Figure 2, we plot $\Delta\Delta G_f$ as a function of R_1 at a crowder concentration of 150 g/L (corresponding to a volume fraction of 0.18). A maximum stabilization is indeed found at $R_1 = 22.2$ Å. Similarly, for a protein with 100 residues, modeled with $R_F = 15$ Å and $R_g = 35$ Å, maximum stabilization is achieved at $R_1 = 16.2$ Å when the crowder concentration is 150 g/L.

On the R_1 axis, the range of applicability of Eq. (7) in calculating the unfolding-state chemical potential is limited from below by the requirement that the first term be less than seven, which corresponds to $R_1 = 16$ Å at $c_1 = 150$ g/L, and from above by the requirement $R_1 \leq R_g$, which is 50 Å in our illustration. A range of crowder radius from 16 to 50 Å at a crowder concentration of 150 g/L is clearly relevant for physiological conditions. As seen earlier, the optimal crowder radius is found within this range. This optimal value increases with increasing crowder concentration, reaching 39 Å at a crowder concentration of 250 g/L (corresponding to $\phi = 0.3$).

We note that the critical reason for the existence of an optimal crowder radius is the fact the second term in Eq. (7) is negative. The negativity of this term is firmly established by theory.[13,14] Thus, even if the value of the optimal crowder radius is determined only approximately, its existence is in little doubt.

Even at the optimal crowder radius of 22.2 Å at a crowder concentration of 150 g/L, the folding stability of the model protein is increased by only 1.3 k_BT or $\sim$0.8 kcal/mol at room temperature. This small magnitude of predicted stabilization by crowding is consistent with both experiments[15–17] and atomistic simulations.[10] As explained previously,[6,8] a contributing factor for the small stabilizing effect is that, in the presence of crowders, the unfolded chain can snake through channels too narrow for the folded protein and occupy two or more interstitial sites simultaneously, thus moderating some of the unfavorable effect of the crowders on the unfolded state. For a crowder concentration of 150 g/L, the stabilization is 1.0 k_BT and 0.7 k_BT, respectively, at $R_1 = 16$ and 50 Å.

Optimal mixing ratio for two species of crowders with different sizes

We mix two species of crowders, with radii of 16 and 50 Å, at different ratios while keeping the total concentration at 150 g/L. In Figure 3, we plot $\Delta\Delta G_f$ as a function of the concentration of the larger crowder. Maximum stabilization is found at an intermediate mixing ra-

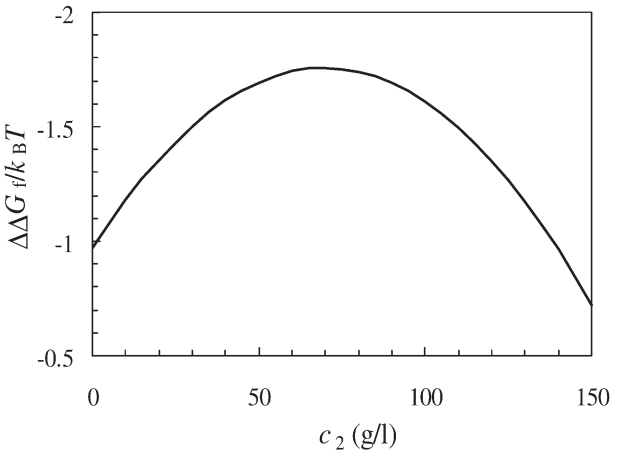

Figure 3

Variation of the crowding effect on folding stability with the mixing ratio of two crowders. The two crowders have radii of 16 and 50 Å. The total crowder concentration is fixed at 150 g/L.

tio. Specifically, at $c_2 = 70$ g/L, the stabilization reaches the maximum of 1.8 k_BT. This value is to be compared with the corresponding values of 1.0 k_BT and 0.7 k_BT, when only the small or larger crowder is present (at the concentration of 150 g/L). In some sense, the mixture of crowders mimics a single species of crowder with an intermediate size. The maximum stabilization is increased when the radius of the smaller crowder is decreased. Unfortunately, the validity of our approximate theory limits the value at which R_1 can be decreased to, which is 16 Å at a total crowder concentration of 150 g/L.

The theoretical results shown in Figure 3 are in qualitative agreement with the experimental results of Liang and coworkers.[2,3] First of all, just like in the experiments, an optimal mixing ratio is found to exist. Second, like seen in the experiments, the increase in stabilization by the mixture relative to the individual species of crowders is modest.

Implications

The results presented above concern the effect of crowding on the folding stability. The folding rate constant can be written in the form

$$k_f = k_0 \exp(-\Delta G^{\ddagger}/k_B T) \qquad (11)$$

where $\Delta G^{\ddagger}$ is the activation free energy for folding and k_0 is a prefactor determined by the dynamics of the protein molecule during folding. If the transition state for folding can be modeled as a compact structure, and additionally the effect of crowding on k_0 is overwhelmed by the effect of crowding on $\Delta G^{\ddagger}$, then the conclusions regarding the effect of crowding on the folding stability can be extended to the folding rate. That is, we expect that the folding rate can be optimized by varying the size of a single crowder and by varying the mixing ratio of two crowders with different sizes.

In the theory presented here, we focus on the sizes of crowders. The shapes of crowders may also significantly influence the effect of crowding. It is very possible that an optimal mixing ratio for crowders with different shapes exists as well.

The models underlying our theory are quite crude. For example, real proteins in the folded state may not be modeled well by a sphere, and a polymer model, especially one that is not self-avoiding, may serve as a poor approximation for the unfolded state. However, even when these complications are accounted for, we still expect $\Delta\mu_F$ and $\Delta\mu_U$, the changes in the chemical potentials of the folded and unfolded states due to crowding, to have dependences on crowder sizes and concentrations very similar to those of Eqs. (3) and (7). Therefore, we believe that the existence of an optimal crowder size and optimal mixing ratio is general.

In conclusion, we have presented a theory for the effect of a mixture of crowders on protein folding, which predicts the existence of an optimal size for a single species of crowders and an optimal mixing ratio for a mixture of different crowders. Based on this prediction, it can be expected that, for any crowding agent (e.g., Ficoll, dextran, and polyethylene glycol), the stabilizing effect can be optimized both by varying the molecular weight and the mixing ratio of two species with different molecular weights. These conjectures await experimental test.

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