



Published in final edited form as:

Proteins. 2008 September ; 72(4): 1109–1113. doi:10.1002/prot.22111.

Effect of Mixed Macromolecular Crowding Agents on Protein Folding

Huan-Xiang Zhou*

Department of Physics and Institute of Molecular Biophysics and School of Computational Science, Florida State University, Tallahassee, FL 32306, USA

Abstract

In cells proteins fold and unfold in the presence of macromolecules with various sizes and shapes. Recent experiments by Liang and co-workers (*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 by varying the mixing ratio of two species with different molecular weights.

Keywords

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 co-workers^{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.

*Correspondence information: e-mail: zhou@sb.fsu.edu; phone: (850) 645-1336; fax: (850) 644-7244.

THEORY

The effect of crowding on a biochemical process is related to the differential effects of crowding on the end states. 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:

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

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

$$\begin{aligned} \Delta \mu_F / k_B T = & -\ln(1 - \varphi) + (1 - \varphi)^{-1} \sum_i \varphi_i (3z_i + 3z_i^2 + z_i^3) \\ & + (1 - \varphi)^{-2} \sum_i \varphi_i z_i \sum_i \varphi_i (9z_i / 2 + 3z_i^2) + 3(1 - \varphi)^{-3} \left(\sum_i \varphi_i z_i \right)^3 \end{aligned} \quad (3)$$

where $k_B T$ is thermal energy, $z_i = R_F / R_i$,

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

is the volume fraction of species i crowder, and $\varphi = \sum_i \varphi_i$. 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 studies⁷ then its change in chemical potential by crowding is given by Equation 3 as well, except that R_F is replaced by an enlarged R_{TS} .

In Equation 4, the crowder concentration is expressed as number density. If the crowder concentration is in g/l, the volume fraction is given by $\varphi_i = 2.52 \times 10^{-3} (R_i^3 / M_i) c_i$, 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 proportion to c_i in g/l. For a protein-like molecule, $\varphi_i \approx 1.2 \times 10^{-3} c_i$, 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, Equation 3 reduces to

$$\begin{aligned} \Delta\mu_f/k_B T = & -\ln(1-\varphi) + (1-\varphi)^{-1}\varphi(3z_1 + 3z_1^2 + z_1^3) \\ & + (1-\varphi)^{-2}\varphi^2(9z_1^2/2 + 3z_1^3) + 3(1-\varphi)^{-3}\varphi^3z_1^3 \end{aligned} \quad (5)$$

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}

$$\Delta\mu_U/k_B T = -\ln(1-\varphi) - \ln S(N) \quad (6)$$

A Gaussian chain is equivalent to the trajectory of a Brownian particle with an effective diffusion constant $D_{\text{eff}} = b^2/6$ (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 Equation 7, which make a positive contribution to $-\ln S(N)$ and are probably negligible when the first term of Equation 7 is $< \sim 7.13$

Combining the last three equations, we have

$$\Delta\mu_U/k_B T = -\ln(1-\varphi) + 3 \sum_i \varphi_i y_i^2 (1 + 2/\pi^{1/2} y_i) - 9 \sum_{i,j} \varphi_i \varphi_j y_i y_j \ln y_{i,j>} \quad (9)$$

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, Equation 9 reduces to

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

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

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 Equation 7 in calculating the unfolding-state chemical potential is limited from below by the requirement that the first term be less than 7, 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 $\varphi = 0.3$).

We note that the critical reason for the existence of an optimal crowder radius is the fact the second term in Equation 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 $1.3k_B T$, or ~ 0.8 kcal/mol at room temperature. This small magnitude of predicted stabilization by crowding is consistent with both experiments¹⁵⁻¹⁷ 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 $1.0k_B T$ and $0.7k_B T$, 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 ratio. Specifically, at $c_2 = 70$ g/l, the stabilization reaches the maximum of $1.8k_B T$. This value is to be compared with the corresponding values of $1.0k_B T$ and $0.7k_B T$, 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 co-workers.^{2,3} First of all, just like in the experiments, an optimal mixing ratio is found to exist. Secondly, 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

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

where ΔG^\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 ΔG^\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 Equations 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 by varying the mixing ratio of two species with different molecular weights. These conjectures await experimental test.

Acknowledgments

This work was supported in part by NIH grant GM058187.

References

1. Zhou H-X, Rivas G, Minton AP. Macromolecular crowding and confinement: biochemical, biophysical, and potential physiological consequences. *Annu Rev Biophys*. 2008 in press.
2. Zhou BR, Liang Y, Du F, Zhou Z, Chen J. Mixed macromolecular crowding accelerates the oxidative refolding of reduced, denatured lysozyme: implications for protein folding in intracellular environments. *J Biol Chem*. 2004; 279:55109–55116. [PubMed: 15494409]
3. Du F, Zhou Z, Mo ZY, Shi JZ, Chen J, Liang Y. Mixed macromolecular crowding accelerates the refolding of rabbit muscle creatine kinase: implications for protein folding in physiological environments. *J Mol Biol*. 2006; 364:469–482. [PubMed: 17027032]
4. Minton AP. Effect of a concentrated “inert” macromolecular cosolute on the stability of a globular protein with respect to denaturation by heat and by chaotropes: a statistical-thermodynamic model. *Biophys J*. 2000; 78:101–109. [PubMed: 10620277]
5. Zhou H-X, Dill KA. Stabilization of proteins in confined spaces. *Biochemistry*. 2001; 40:11289–11293. [PubMed: 11560476]
6. Zhou H-X. Protein folding and binding in confined spaces and in crowded solutions. *J Mol Recognit*. 2004; 17:368–375. [PubMed: 15362094]
7. Hayer-Hartl M, Minton AP. A simple semiempirical model for the effect of molecular confinement upon the rate of protein folding. *Biochemistry*. 2006; 45:13356–13360. [PubMed: 17073456]
8. Zhou H-X. Protein folding in confined and crowded environments. *Arch Biochem Biophys*. 2008; 469:76–82. [PubMed: 17719556]
9. Elcock AH. Atomic-level observation of macromolecular crowding effects: escape of a protein from the GroEL cage. *Proc Natl Acad Sci USA*. 2003; 100:2340–2344. [PubMed: 12601146]
10. Cheung MS, Klimov D, Thirumalai D. Molecular crowding enhances native state stability and refolding rates of globular proteins. *Proc Natl Acad Sci USA*. 2005; 102:4753–4758. [PubMed: 15781864]
11. Cheung MS, Thirumalai D. Effects of crowding and confinement on the structures of the transition state ensemble in proteins. *J Phys Chem B*. 2007; 111:8250–8257. [PubMed: 17585794]
12. Lebowitz JL, Helfand E, Praestgaard E. Scaled particle theory of fluid mixtures. *J Chem Phys*. 1965; 43:774–779.
13. Szabo A, Zwanzig R, Agmon N. Diffusion-controlled reactions with mobile traps. *Phys Rev Lett*. 1988; 61:2496–2499. [PubMed: 10039133]
14. Berezhkovskii AM, Makhnovskii YA, Weiss GH. Trapping of Brownian particles by random spheres of different radii. *J Chem Phys*. 2001; 115:5376–5380.
15. Qu Y, Bolen DW. Efficacy of macromolecular crowding in forcing proteins to fold. *Biophys Chem*. 2002; 101–102:155–165.
16. Spencer DS, Xu K, Logan TM, Zhou HX. Effects of pH, salt, and macromolecular crowding on the stability of FK506-binding protein: An integrated experimental and theoretical study. *J Mol Biol*. 2005; 351:219–232. [PubMed: 15992823]
17. Roberts A, Jackson SE. Destabilised mutants of ubiquitin gain equal stability in crowded solutions. *Biophys Chem*. 2007; 128:140–149. [PubMed: 17434659]

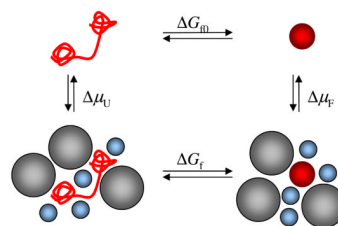


Fig. 1. Thermodynamic cycle for obtaining the effect of crowding on the folding stability. ΔG_{f0} and ΔG_f , respectively, are the folding free energies in the absence and presence of crowding. $\Delta\mu_U$ and $\Delta\mu_F$ are the changes in the chemical potentials of the unfolded and folded states, respectively, due to crowding.

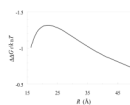


Fig. 2. Variation of the crowding effect on folding stability with the crowder size. A single species of crowder, at a concentration of 150 g/l, is present.

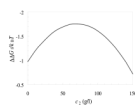


Fig. 3. Variation of the crowding effect on folding stability with the mixing ratio of two crowders. The two crowders have radii of 16 and 50 Å. The total crowder concentration is fixed at 150 g/l.