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A simple quantitative model of macromolecular crowding effects on protein folding: Application to the murine prion protein(121–231)

Fernando Bergasa-Caceres *, Herschel A. Rabitz

Department of Chemistry, Princeton University, Princeton, NJ 08544, United States

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ABSTRACT

A model of protein folding kinetics is applied to study the effects of macromolecular crowding on protein folding rate and stability. Macromolecular crowding is found to promote a decrease of the entropic cost of folding of proteins that produces an increase of both the stability and the folding rate. The acceleration of the folding rate due to macromolecular crowding is shown to be a topology-dependent effect. The model is applied to the folding dynamics of the murine prion protein (121–231). The differential effect of macromolecular crowding as a function of protein topology suffices to make non-native configurations relatively more accessible.

1. Introduction

Theoretical and experimental findings show that protein folding dynamics are significantly influenced by macromolecular crowding conditions similar to those likely to exist *in vivo* [1–17]. Moreover, evidence shows that macromolecular crowding *in vitro* dramatically accelerates amyloid formation in several fibril-forming proteins including the human prion protein [1,2] and α-synuclein [18,19] a protein whose dynamics have been associated with Parkinson's disease [20]. On the basis of these findings it has been suggested that the effects of macromolecular crowding on protein folding dynamics might play a significant role in promoting the onset of several neurodegenerative diseases, including prion-related proteinopathies. Thus, developing and applying dynamical models to protein folding under crowded conditions has become a pressing issue in biophysical chemistry.

This Letter employs a previously developed model for protein folding kinetics [21,22] to quantify the entropic effects of macromolecular crowding on the stability and folding rate of proteins. Macromolecular crowding is shown by the model to increase the stability and the folding rate of proteins in agreement with experimental results. Two significant effects on protein folding of macromolecular crowding are quantitatively studied: (a) the increase in the stability of the folded structure, and (b) the acceleration of the folding rate. Moreover, the acceleration effect is found to be dependent on contact order, with the acceleration being larger for proteins with higher contact order in the folded structure. The model is then applied to study the effects of macromolecular crowding on the extremely fast, apparently two-state, folding transition of the C-terminal domain of the murine prion protein, *m*PrP (121–231) [23]. Both effects are shown to be strong enough to play a role in the pathogenic interconversion of the murine prion protein, *m*PrP (121–231). The significance of these findings on the pathogenic mechanism of the prion protein will also be discussed.

2. The murine prion protein (121–231)

2.1. Structure

Murine prion protein PrP^C is a glycoprotein of 209 amino acids, (numbered 23–231 according to human PrP) attached to the cell membrane by a glycosylphosphatidyl inositol (GPI) anchor at ser231 [22,23]. Its three-dimensional structure includes an autonomously folding C-terminal domain including residues 121–231, *m*PrP (121–231) [23]. The structure of the C-terminal domain of PrP^C includes up to three α-helices and a short two-stranded antiparalell β-sheet. A high resolution structure of PrP^C is still unavailable. On the basis of the NMR structure (PDB code: 1AG2), the relative contact order of *m*PrP (121–231) was estimated to be RCO = 0.104 [24].

2.2. Folding kinetics

The folding kinetics of *m*PrP (121–231) have been explored employing a mutant F175W in order to have a fluorescence probe [23]. The folding reaction is very fast, with a half-life of $\tau \sim 170$ μs at 4 °C. The reaction proceeds in apparent two-state fashion, being one of the fastest naturally occurring protein folding reactions known to date [23]. Recent experimental evidence on human prion protein (90–231) *hu*PrP(90–231) suggests that there might be

* Corresponding author.
E-mail addresses: f_bergasa@yahoo.es (F. Bergasa-Caceres), Hrabitz@Princeton.edu (H.A. Rabitz).

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intermediates along the folding pathway of prion proteins [25,26], but the evidence is limited and *hu*PrP(90–231) is a significantly longer protein than *m*PrP (121–231). There is also some evidence for a very early intermediate in *m*PrP (121–231) [26]. This intermediate would be only marginally stable and involve few native-like contacts, with most of the chain folding in a two-state collapse-like mode. The assumption will thus be made in this Letter that the folding kinetics of *m*PrP (121–231) can be adequately analyzed through a two-state model.

3. The folding model

3.1. Dependence of two-state folding rates upon native topology and the entropic cost of folding

The analysis presented in this Letter relies on an equation developed in previous work [21,22] to study the kinetic rate κ_f of collapse-like folding transitions:

$$\ln \kappa_f \approx \ln g - \mathrm{RCO}(\mathbf{\Gamma})\Delta G_{\mathrm{conf,Nat}}/(k\mathrm{T}) \quad (1)$$

where g is the system's diffusional frequency, $\mathrm{RCO}(\mathbf{\Gamma})$ is the relative contact order [27], and $\Delta G_{\mathrm{conf,Nat}}$ is the conformational entropic free energy change associated with the folding transition. The thermodynamic conformational entropic free energy cost of folding a protein of length L, $\Delta G_{\mathrm{conf,Nat}}$, can be approximately written as

$$\Delta G_{\mathrm{conf,Nat}} \approx -k\mathrm{T}\, L \ln(f/f_0) \quad (2)$$

where the average conformational freedom is f for an amino acid in the compact state after the collapse, f_0 is the average conformational freedom per residue in the unfolded state [21,22], and L is the length of the collapsing region. Eq. (1) implies that the logarithm of the collapse rate should decrease linearly with the contact order of the native topology times the entropic configurational cost of folding.

Eq. (2) bears a striking resemblance to that observed experimentally for two-state proteins [27] that yields [28]

$$\ln \kappa_{\mathrm{coll}} \approx 16.1 - \mathrm{RCO}(\mathbf{\Gamma})71 \quad (3)$$

This equation is equivalent to Eq. (1) if we set $\langle \Delta G_{\mathrm{conf,Nat}} \rangle \approx 71$ kT, where $\langle \Delta G_{\mathrm{conf,Nat}} \rangle$ is the average of $\Delta G_{\mathrm{conf,Nat}}$ over a sample of proteins with two-state kinetics, and $g \approx 10^7\ \mathrm{s}^{-1}$. A value of 71 kT for $\langle \Delta G_{\mathrm{conf,Nat}} \rangle$ is reasonable for proteins of ~70–100 amino acids with ~1/3 hydrophobic amino acids, each contributing ~3–5 kT to the hydrophobic stabilization term ΔG_{hyd}, for an overall stabilization energy of ~10–20 $k\mathrm{T} \approx \Delta G_{\mathrm{hyd}} + \Delta G_{\mathrm{conf,Nat}}$. A value of $g \approx 10^7\ \mathrm{s}^{-1}$, consistent with current estimates of the limiting protein folding speed limit, is equivalent to folding in the absence of a significant entropic barrier (i.e., $\Delta G_{\mathrm{conf,Nat}} \approx 0$) [29].

For relatively concentrated protein solutions, excluded volume effects are dominant when analyzing the effect of macromolecular crowding [4–6] on protein folding rates and stability [15]. When concentrations become significantly higher, other factors can enter including increased viscosity and protein polarity which may become dominant and compensate for the increase in folding rates and stability due to macromolecular crowding [15]. The main goal of the Letter is to determine whether macromolecular crowding effects due to excluded volume are influenced by protein topology. In addition, we provide a quantitative model of those effects for the concentration regime in which they are dominant and apply the model to *m*PrP (121–231).

3.2. Effects of macromolecular crowding on stability

As the concentration C of macromolecules in the protein solution increases, whether through the addition of more protein molecules or other macromolecular species, the available volume per molecule decreases [4–6] and steric interactions between neighboring molecules become more significant. Then, the conformational freedom of the unfolded protein chain f_0 decreases, due to the reduction in available space for the side chains in the unfolded state. As long as the solution remains sufficiently dilute, this excluded volume effect is negligible, $\partial f_0/\partial C \approx 0$. As the bulk concentration increases, steric hindrance will become progressively more important and for a concentrated solution we expect $\partial f_0/\partial C < 0$.

The native state conformational freedom f is less affected by macromolecular crowding related excluded volume effects because a large number of the amino acids in the folded state are densely packed inside the protein globule and much less exposed to conditions in the bulk, the approximation will then be made here that $\partial f/\partial C \approx 0$. Then, a decrease in f_0 implies a decrease in the entropic cost of folding $\Delta G_{\mathrm{conf,Nat}} \approx -kTL\ln(f/f_0)$, as long as $\partial(f/f_0)/\partial \mathrm{C} > 0$. Thus, in accordance with existing theoretical approaches [17], we have:

$$\Delta G_{\mathrm{conf,Nat}}(\mathrm{concentrated}) > \Delta G_{\mathrm{conf,Nat}}(\mathrm{dilute}) \quad (4)$$

The overall free energy change upon folding can be written as $\Delta G_{\mathrm{Nat}} = \Delta G_{\mathrm{conf,Nat}} + \Delta G_{\mathrm{int,Nat}}$, where $\Delta G_{\mathrm{int,Nat}}$ is the free energy change upon formation of the stabilizing enthalpic interactions. The enthalpic stabilizing interactions can be treated as independent of the bulk concentration, as they are not dependent on steric interactions with neighboring macromolecules as long as the solution remains dilute enough. Then, as the solution goes from a dilute to a concentrated state, the change in free energy of folding $\Delta\Delta G_{\mathrm{Nat,dil}\to\mathrm{conc}}$, can be written as:

$$\Delta\Delta G_{\mathrm{Nat,dil}\to\mathrm{conc}} \approx \Delta\Delta G_{\mathrm{conf,Nat,dil}\to\mathrm{conc}} \approx -k\mathrm{T}\, L\, \ln(f_{0,\mathrm{conc}}/f_{0,\mathrm{dil}}) > 0 \quad (5)$$

here $f_{0,\mathrm{dil}}$ is the conformational freedom of the unfolded chain in a dilute solution and $f_{0,\mathrm{conc}} < f_{0,\mathrm{dil}}$ is the conformational freedom of the unfolded chain under concentrated conditions. From Eq. (5) it is clear that increasing levels of macromolecular crowding will increase the stability of the native state as the macromolecular bulk concentration increases, once again in agreement with existing theoretical approaches [17].

3.3. Effects of macromolecular crowding on the folding rate

Inspection of Eq. (1) shows that a decrease in the entropic cost of folding $\Delta G_{\mathrm{conf,Nat}}$ implies a decrease in the two-state folding rate. Thus, the change in folding rate between a dilute and a concentrated solution with $f_{0,\mathrm{conc}} < f_{0,\mathrm{dil}}$ can be expressed from Eqs. (1) and (5) as:

$$\ln(\kappa_{\mathrm{f,conc}}/\kappa_{\mathrm{f,dil}}) \approx -\mathrm{RCO}(\mathbf{\Gamma})\, L\, \ln(f_{0,\mathrm{conc}}/f_{0,\mathrm{dil}}) > 0 \quad (6)$$

such that $\kappa_{\mathrm{f,dil}} < \kappa_{\mathrm{f,conc}}$. This equation implies that macromolecular crowding induces an acceleration of the protein two-state folding rate. Importantly, inspection of Eq. (6) shows that the change in the free energy of folding due to increasing concentration does not depend on the protein's topology, while the acceleration of folding rates directly depends on the overall topology of the folded state represented by $\mathrm{RCO}(\mathbf{\Gamma})$.

3.4. When is a protein solution concentrated?

Based on the analysis presented above, a prime issue is the level of macromolecular crowding at which the solution should be considered concentrated. A macromolecular solution will be concentrated when the available bulk volume per macromolecule becomes comparable with molecule size. An estimate of the size of an unfolded 111 residue protein, such as *m*PrP (121–231) can be performed employing computational results showing that the radius of gyration of an unfolded protein of such length should