Available online at www.sciencedirect.com
SCIENCE DIRECT
Polymer 47 (2006) 2564–2570
polymer
www.elsevier.com/locate/polymer

Depletion force from macromolecular crowding enhances mechanical stability of protein molecules

Guanghui Ping [1], Guoliang Yang, Jian-Min Yuan *

Department of Physics, Drexel University, Philadelphia, PA 19104, USA

Received 18 October 2005; received in revised form 3 December 2005; accepted 5 December 2005
Available online 28 February 2006

Abstract

In crowded solutions the presence of many cosolutes often affects the stability of compact polymers, such as globular proteins. Important examples of crowded environments are those inside some cells, where protein stability or aggregation rates are affected by the presence of co-existing bio-macromolecules. In the present article the concept of depletion force from colloidal physics and theoretical techniques developed for polymer science have been applied to study the effects of macromolecular crowding on protein stability. A continuous three-dimensional polymer model is used to simulate the behavior of protein under the conditions of macromolecular crowding and the depletion force based on such a model is calculated. Calculated results have been compared with the measured results in our laboratory, where the enhancement of the forces required to unfold ubiquitin molecules in a solution crowded with dextran has been measured using single-molecule atomic force microscopy techniques. Comparison between the calculated results and experimental observations shows that only qualitative agreement has been reached in the sense that both show a larger force required because of crowding as a protein molecule is mechanical stretched, but the magnitude of the enhancement of the unfolding force theoretically predicted is small compared to the measured value. Possible sources of discrepancy and improvements of the model are discussed.
© 2006 Elsevier Ltd. All rights reserved.

1. Introduction

The study of macromolecular crowding effects on protein properties has a long history [1–3] and has simulated revival of activities [4–6] due to current interests on protein aggregations as a potential cause for neurodegenerative diseases. This is because the cellular environments are often packed with other biomolecules and this crowdedness may affect the stability and aggregation rates of proteins inside cells. To simulate the crowded environments in vitro, scientists often add inert crowding agents to protein solutions. However, up to now, all experimental investigations on macromolecular crowding effects are based on measurements of bulk properties of protein solutions [1–7]. In a recent experiment in our laboratory [8], single-molecular techniques based on atomic force microscopy (AFM) are employed to measure directly the effects of macromolecular crowding on the mechanical force required to unfold a single protein molecule. Details of our experiment are reported somewhere else [8] and the purpose of the present article is to present a theoretical treatment of the subject, including the theoretical formulation, numerical simulations and calculations of the enhancement of the mechanical unfolding force due to macromolecular crowding.

Our theoretical approach is developed based on the idea of depletion force, which is a concept widely used in colloidal and soft-matter physics [9–13]. The theory of depletion force was first developed by Asakura and Oosawa [9] in the late 1950s and rediscovered decade later by Vrij [10]. Briefly stated, the theory predicts the existence of an effective attractive force acting between two hard particles suspended in a solution which contains many cosolute molecules. This force arises from an overall gain of entropy of this composite system. When two suspended hard particles are in contact with each other, the cosolute molecules gain more space, in which they can freely move, therefore, the entropy of the overall system is higher than that when the two hard particles are far apart.

In the application to the studies of macromolecular crowding effects on protein aggregations, the prediction of the depletion force theory can be briefly described as follows: in a solution in which protein co-exists with other macromolecules, we assume that no direct interactions exist between two protein molecules, between protein and crowder molecules, or between two crowder molecules. That means, for simplicity in

* Corresponding author.
E-mail address: Yuanjm@drexel.edu (J.-M. Yuan).
[1] Present address: Genome and Biomedical Science Facility, University of California, 451 East Health Science Drive, Davis, CA 95616-8816, USA

0032-3861/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.polymer.2005.12.085

presenting the essentials, we assume that both protein and crowding molecules can be represented by hard spheres with radii given by R_p and R_c, respectively, for protein and crowders. Then associated with each protein molecule is a depletion zone (V_{ex}) of the size of $(4\pi/3)(R_p + R_c)^3$, inside of which the centers of mass of any crowder molecules can not get into. The entropy of the crowder molecules increases with the volume within which they can freely move. This free volume is equal to $V - V_{ex}$, where V is the volume of the solution. As shown in Fig. 1, let us consider the case where two protein molecules co-exist with many crowder molecules in solution. The free volume of the crowders is largest when the protein molecules are in direct contact with each other, because the depletion zone of the two protein molecules is smallest due to overlapping of their depletion zones. Since there are many crowders in solution, the entropy of the entire system is largest when protein molecules are in direct contact. According to the second law of thermodynamics, a spontaneous process takes place in the direction of increasing entropy, the net effect is that the two protein molecules tend to form an aggregate, as if an attractive force exists between the protein molecules. This type of depletion forces has been measured in colloidal and soft-matter physics experiments (e.g. Ref. [11]) and also in protein and DNA solutions [12,13]. This is consistent with the prediction of Minton and others based on thermodynamics and the scaled particle theory that the effect of macromolecular crowding tends to enhance the stability of protein aggregates by increasing the rate of protein aggregation, relative to the rate of dissociation [4,7,14,15].

Related to the properties of a single protein molecule, one may ask what the theory of depletion force would predict, in particular, about the effects of macromolecular crowding on the folding and unfolding properties of the protein. One of the objectives of the present article is to develop such a theoretical treatment, in which it is predicted that based on the concept of depletion force the mechanical force required to unfold a protein molecule increases due to macromolecular crowding. Furthermore, numerical simulations and calculations will be carried out to give an estimate of the amount of change in mechanical force and the results are compared with the measured values obtained in our laboratory. The goal is to shed light on the measured values of the enhancement factor observed in the single-molecule mechanical unfolding experiment mentioned above.

Theoretical treatments of the effects of macromolecular crowding and confinement on protein aggregation and folding properties have a long history. Since 1980s, Minton and co-workers have studied these effects using thermodynamics and the scaled particle theory for fluid mixtures [4,7,14–16]. Simple polymer models, such as a Gaussian chain, and stochastic dynamics have been used in recent studies by Zhou [17,18] and self-avoiding polymer models used by Minton [19]. Stimulated by sol–gel experimental results of Wei and co-workers [20,21], Ping et al. [20–22] have employed a two-dimensional HP model [23] to study the effects of confinement and macromolecular crowding on protein stability and folding/unfolding dynamics. An off-lattice 46 bead model was used in a molecular dynamics study of the effects of confinement and crowding by Friedal et al. [24]. Similarly, detailed simulations using molecular dynamics based on more realistic models for β-hairpin or β-sheet proteins have been carried out in the studies of confinement [25] and macromolecular crowding effects [26] by Thirumalai and co-workers. Harries and Parsegian, on the other hand, have studied the effects of small cosolutes on protein folding using Monte Carlo simulations based on a grand canonical ensemble approach [27]. Furthermore, Kinjo and Tanaka [28] have studied macromolecular crowding effects using density functional theory for fluids. The present approach is different from the above ones, but more along the line of polymer models [17,19].

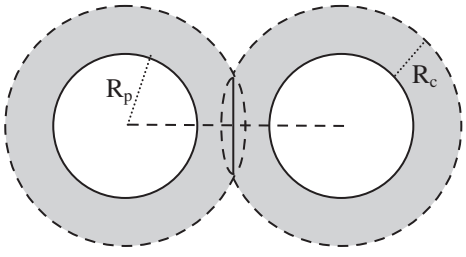

Fig. 1. The depletion zones surrounding two hard spheres. The shaded region denotes the depletion zone. The inner sphere has a radius of R_p and the outer shell is of the thickness which equals the radius of the crowder molecule, R_c.

The organization of the article is as follows: we briefly review our experimental work on the enhancement of mechanical unfolding force due to crowding measured using AFM in Section 2. The theoretical development in applying the concept of depletion force to protein folding and unfolding will be described in Section 3. This formulation and some techniques for polymer science are applied to an off-lattice three-dimensional (3D) polymer model for a protein molecule in the calculations of the additional unfolding force contributed by crowding in Section 4 and the results are presented in Section 5. This is followed by a discussion and conclusion section.

2. Brief review of experimental results

The experimental measurements in our lab of the macromolecular crowding effects on the mechanical unfolding of protein molecules were carried out using a modified atomic force microscope [29]. The sample used was ubiquitin molecules in an N–C linked octamer synthesized via protein engineering [30], and crowding agent was dextan molecules with an average molecular weight of 40 kDa (Sigma) and an average hydrodynamic radius of 3.5 nm [31]. Ubiquitin has been used widely as a model system for protein folding studies, and it has also been successfully utilized recently for single molecule measurements [30,32,33]. Dextran was chosen as the crowding agent since dextran is inert and highly soluble, and has been used in many experimental studies of the macromolecular crowding effects. The details of the experiments are reported elsewhere [8]. Briefly, the ubiquitin polymer was dissolved in PBS buffer with a protein concentration of 50 μg/ml, and 20 μl of the protein solution was deposited on a fresh gold surface. While both the sample and the AFM tip

immerged in the solution, individual ubiquitin polymers can be tethered between the tip and the gold surface via non-specific interactions, and mechanical unfolding can be induced by stretching the polymerized molecules. During an experiment, the protein molecules remain attached to the surface, therefore the same batch of molecules on the gold surface can be studied in different solution conditions by flushing the liquid chamber with the desired solution. For our macromolecular crowding studies, dextran (dissolved in PBS buffer) solutions with concentrations of 0, 100, 200 and 300 g/l were used.

Fig. 2 shows two 'force curves' obtained by pulling ubiquitin polymers at dextran concentrations of 0 and 200 g/l, respectively. Each peak in the sawtooth patterns represents the unfolding of a single protein molecule. The irregular peaks at the beginning of curves are due to the non-specific interactions between the tip and the gold surface. By fitting rising part of each peak to the worm-like-chain model, as shown in Fig. 2, the contour length increase from a unfolding event was obtained, which serves as one of the parameters to verify that the observed peaks are from unfolding of individual protein molecules. The unfolding force for each ubiquitin molecule is not the same, because thermal fluctuation plays an important role in such single molecule experiments. The unfolding rates in the absence of an applied force can be obtained from a Monte Carlo simulation [30].

The effects of macromolecular crowding on the mechanical unfolding of ubiquitin were determined by measuring the unfolding forces at four different concentrations of dextran. Fig. 3 shows that the average unfolding force changes from 166 to 201 pN as the dextran concentration increases from 0 to 300 g/l at a pulling rate of 50 nm/s, corresponding to a force loading rate of 4.2 nN/s. This increase in unfolding forces corresponds to a reduction of the zero-force unfolding rate by a factor of 6.2 [8]. It would be interesting to provide a theoretical base for the understanding of the phenomenon observed.

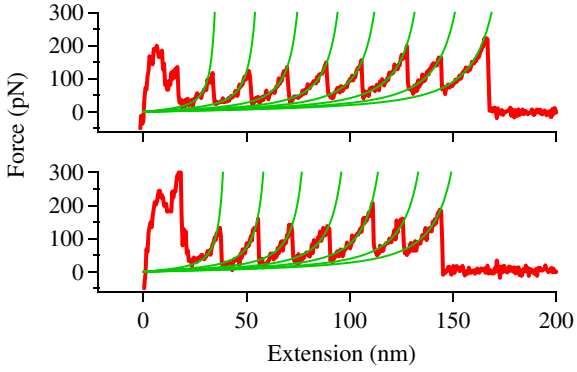

Fig. 2. Force vs. extension curves obtained from stretching ubiquitin polymers in the presence of 0 (top) and 200 g/l (bottom) of dextran in PBS buffers. The force loading rate used for these curves was 4.2 nN/s (pulling speed = 50 nm/s). The rising parts of the force peaks are fitted to the WLC model. The persistence length used in the fitting was 0.40 ± 0.02 nm. The contour length increment between adjacent peaks was found to be $\Delta L = 24.9 \pm 2.4$ nm ($n = 676$), which is in consistent with the expected value of 24.4 nm from the structure of ubiquitin [30]. The values of the persistence length and that of ΔL were found not to be dependent on the dextran concentration. An automatic fitting procedure was developed to fit the force curves with two adjustable parameters [8], and it was found that the values of ΔL and the persistence length remain the same at different dextran concentrations.

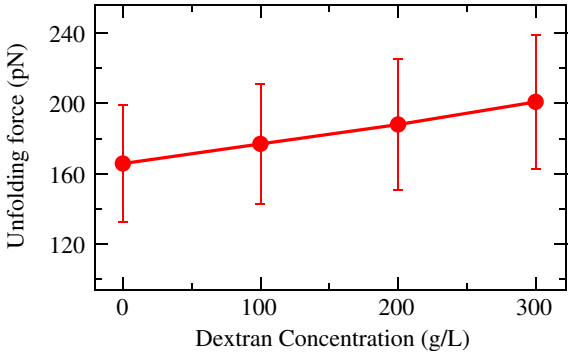

Fig. 3. Dependence of the unfolding forces of ubiquitin on the dextran concentration at a force loading rate of 4.2 nN/s. Each point in the plot is the average of a number of data points ($n = 151$, 137, 148 and 207, respectively) with standard deviations of 33, 34, 37 and 38 pN, respectively.

3. Application of depletion force concept to the effects of macromolecular crowding on protein folding

As mentioned earlier, the concept of depletion force was first introduced by Asakura and Oosawa [9] as a force acting between two suspended colloidal particles in a solution of macromolecules. In the presence of N macromolecules a force acting between two colloidal particles can be expressed as a differential of the canonical partition function with respect to the inter-particle distance a,

$$F_{\mathrm{d}} = Nk_{\mathrm{B}}T\frac{\partial \ln Q(V,T)}{\partial a} \tag{1}$$

where $Q(V,T)$ is a single-particle (referring to the macromolecules here and to the macromolecular crowders later for our purpose) partition function and a is inter-particle distance, V is the total volume of the solution, and T the temperature of the system. For a system at constant T and V, the momentum space part of the partition function is independent of a and only the configuration space part, Q_{r}, of the partition function needs to be included in $Q(V,T)$. Q_{r}, on the other hand, is given by

$$Q_{\mathrm{r}} = \int_V \mathrm{e}^{-\beta V(\vec{r},a)}\mathrm{d}^3 r \tag{2}$$

where $V(\vec{r},a)$ is the potential energy of a macromolecule located at $\vec{r}$ when two colloidal particles are separated by a distance a. Since it is assumed that no interaction potentials exist between macromolecules, between colloidal particles, or between a macromolecule and a particle, Q_{r} is simply equal to the volume available to a macromolecule, which is equal to $V - V_{\mathrm{ex}}$, where V_{ex} is the excluded volume, given by the total volume of the depletion zones. Then the depletion force, F_{d}, is given approximately by

$$F_{\mathrm{d}} = -\left(\frac{Nk_{\mathrm{B}}T}{V}\right)\frac{\partial V_{\mathrm{ex}}}{\partial a} \tag{3}$$

Using the van't Hoff formula for dilute solutions, one can write Eq. (3) in the form

$$F_d = -p_{os}\frac{\partial V_{ex}}{\partial a} \quad (4)$$

where p_{os} is the osmotic pressure due to the macromolecules. For two colloidal particles it was further showed that the depletion force, the effective attractive force acting between two particles, could be written as

$$F_d = -p_{os}A \quad (5)$$

where A is the maximal cross-section area of the overlapping depletion zone when two colloidal particles are close to each other such that the closest distance between the surfaces of two spherical particles is within a distance of the diameter of a macromolecule.

In the form of Eq. (5), the depletion force has a simple physical interpretation, that is, it is a force due to the imbalance of the osmotic pressure acting on the surfaces of two colloidal particles, when they are close to each other by a distance less than the diameter of a macromolecule. Eq. (5) was also derived by Vrij [10]. This form, or equivalently Eq. (4), also has an immediate generalization where the concentration of the macromolecules is high so that the van't Hoff formula is not valid. Eq. (4) is the expression of the depletion force which will be used to calculate the change of mechanical force required to unfold a protein molecule due to the presence of macromolecular crowders. The force is non-zero, because as protein unfolds from its native structure (roughly spherical shape) its depletion zone increases due to the deformation of the molecule. For the process of protein unfolding the distance parameter in Eq. (4) is replaced by a reaction coordinate, such as the end-to-end distance or the radius of gyration. The reason that the sign in Eqs. (4) or (5) is negative is because it is an effective attractive force acting between two colloidal particles. The negative sign for a protein folding process means that the depletion force acts to push the protein to assume the most compact structure, that is, the structure with the least depletion zone. Thus, it acts to enhance the stability of the native structure. This force is entropic in origin, because for simplicity it is derived by assuming that the particles and macromolecules are hard spheres. This force must then arise from entropic part of the Helmhotz free energy. The entropy of the whole system increases as the macromolecules gain free space in which they can move.

4. Numerical simulations based on a 3D polymer model

The depletion force causes an increase in the force required to unfold a protein in solutions crowded with macromolecules. The results of numerical simulations to calculate the depletion force are presented in this section. The intention is to simulate the experimental results described in Section 2.

In order to calculate the depletion force using Eq. (4), an expression of the osmotic pressure corresponding to the dextran solution and a relation between V_{ex} and the end-to-end distance, S, are needed. Osmotic pressure can be approximated by expressions derived based on the scaled particle theory [34–36]. The functional relation between V_{ex} and S can be calculated in principle using all-atom molecular dynamics simulation under mechanical stretching at a constant pulling speed. However, the experimental pulling speed is too slow, i.e. the time scale is too long, to simulate realistically using currently available methods. The calculation of the surface area and the associated depletion zone (excluded volume) of a real protein molecule is another challenging problem. As mentioned above, polymer models [4,17,19] as well as lattice models [20–22] are often used in the studies of macromolecular crowding and confinement. More recently, molecular dynamics has been used [23–25] in these simulations as well. In the present article we shall find a functional relation based on simulations in terms of an off-lattice three-dimensional (3D) polymer model [22]. The goal is to provide guides to qualitative behaviors of protein–crowder systems.

The 3D polymer model consists of 76 monomers, each corresponding to an amino acid residue of ubiquitin. Each monomer is represented by a hard sphere (bead) of the size, $R_m = 0.125$ nm and the bond length between two neighboring monomers (bead center to bead center) is $b = 0.34$ nm. A dextran molecule is represented by a hard sphere of size, R_c, determined by the molecular weight of the dextran molecules.

To find a statistical relation between the excluded volume and the end-to-end distance, one needs a large number of polymer conformations. Since the total number of conformations is astronomically large, a Monte Carlo (MC) process is used to generate a random sampling of conformations. One way to carry out such a sampling process is to start with conformations of a self-avoiding polymer and then use a pivot algorithm to generate as many random conformations as one needs. The starting conformations are, however, obtained through a 3D growth algorithm. A pivot/growth algorithm developed in polymer sciences [37,38] works as follows:

1. Grow a self-avoiding conformation of the 76mer or initiate with the straight-rod conformation. The latter is mainly aimed at obtaining structures with extended conformations, for they are more time-consuming to obtain using a growth algorithm.
2. Choose randomly a bead along the polymer chain. This bead separates the polymer into a short and a long segment. Rotate the short segment about the pivot bead through a triplet of angles (α, β, γ) along the x-, y-, and z-axes. These axes are arbitrarily chosen and, once chosen, are fixed in the space. The angles are randomly selected within a range, which is empirically determined to be within $-0.5° < \text{angle} < 0.5°$.
3. Once a self-avoiding conformation is obtained, accept it and proceed with the excluded volume calculation.
4. Repeat Steps 2–3 to generate the next conformation.
5. Repeat Steps 1–4 for enough MC steps, until good statistics are collected.

For a given conformation obtained using the pivot/growth algorithm, its excluded volume is calculated using another Monte Carlo procedure, described as follows:

1. For this conformation, find a minimal rectangular parallelepiped enveloping the depletion zone of the polymer completely.
2. Choose randomly a point inside the rectangular parallelepiped; if the distance of this point and the center of any monomer is less than the sum of the monomer radius and crowder radius ($R_m + R_c$), then it will be counted; otherwise it will not.
3. After a large number of random points are drawn, approximate the excluded volume by the product of the box volume and the count ratio.

5. Results of numerical simulations

The methods outlined in the previous sections have been applied to the 76mer in a solution of macromolecular crowders and the results of the calculated excluded volume are presented in Fig. 4, which show that V_{ex} is an increasing function of both the end-to-end distance S of the polymer and the size of crowders. About 10,000 self-avoiding chains were generated by the growth mechanism and for each chain 2000 conformations were obtained by pivot rotations, thus in total 2×10^7 conformations were studied for its V_{ex} and S to obtain a single curve in Fig. 4. Since S is binned into 2000 values in each curve, on the average each V_{ex} is an average of 10,000 conformations. This is, of course, true only in the average sense, for the probability distribution of the end-to-end distance follows a Gaussian distribution. Fig. 4 also indicates that the functional relation between the excluded volume and the end-to-end distance shows a qualitative change of behavior at about 7 nm. As a result, curves in Fig. 5 discussed below peak around 7 nm as well.

For the calculation of the depletion force based on Eq. (4), the derivative $\partial V_{ex}/\partial a$ is needed, which can be obtained by a linear fit to a small segment of a curve such as those presented in Fig. 4. The slope of the fitting line gives the derivative needed. The osmotic pressure factor appearing in Eq. (4), on the other hand, can be approximated by an expression obtained

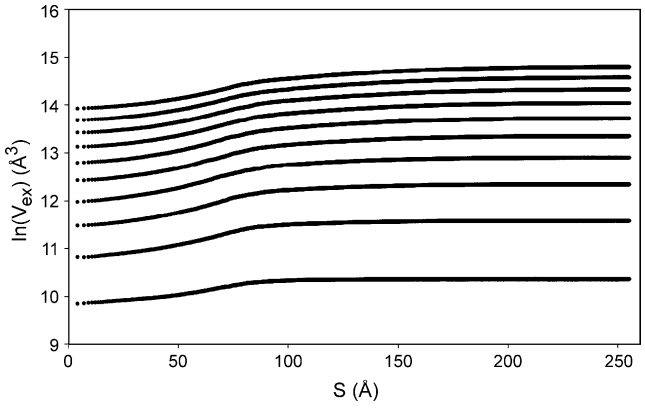

Fig. 4. Natural logarithm of excluded volume, $\ln(V_{ex})$ as a function of the end-to-end distance, S, of the 3D off-lattice 76mer. S is in unit of 0.1 nm and V_{ex} in nm^3. In the order from bottom up, the 10 curves correspond to simulations for crowders of size 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 4.0, 4.5, and 5 nm.

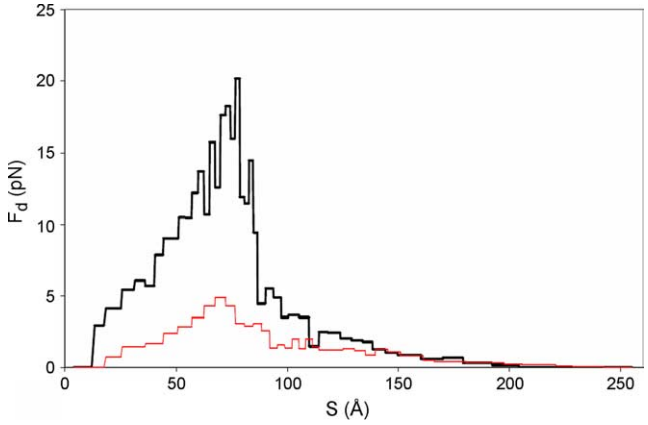

Fig. 5. Depletion force-extension profile. Shown here are curves for crowders of size 1 nm (thicker line) and 3 nm (thinner line), respectively. The depletion force, F_d, is in unit of 1 pN and end-to-end extension, S, in unit of 0.1 nm. The volume fraction is fixed at 40% and temperature is set at 300 K.

using the scaled particle theory for fluid mixtures [33–35]. The expression states that

$$p_{os} = \rho k_B T \frac{1+\varphi+\varphi^2}{(1-\varphi)^3} \tag{6}$$

where φ is the volume fraction and ρ the number density of the macromolecular crowders in solution. Two examples of the depletion force curves calculated based on Eqs. (4) and (6) are presented in Fig. 5, which show that depletion force first rises, then decreases, with S and peaks around $S=7$ nm.

To see how depletion force depends on the volume fraction of the macromolecular crowders, the maximal depletion force, such as those peaks in Fig. 5, is plotted as a function of the volume fraction in Fig. 6. This figure shows that the depletion force increases with the volume fraction in a curve rising faster than a linear dependence. This is to be compared with the experimental results shown in Fig. 3, obtained by averaging over many data collected. Although in both cases depletion force increases with the crowder concentration, the theoretical curve of Fig. 6 predicts a faster rising trend than that observed experimentally. Furthermore, the magnitude of theoretical predicted value ($\sim$4 pN) at $\varphi=0.3$ is about one order magnitude smaller than the observed value ($\sim$35 pN) at

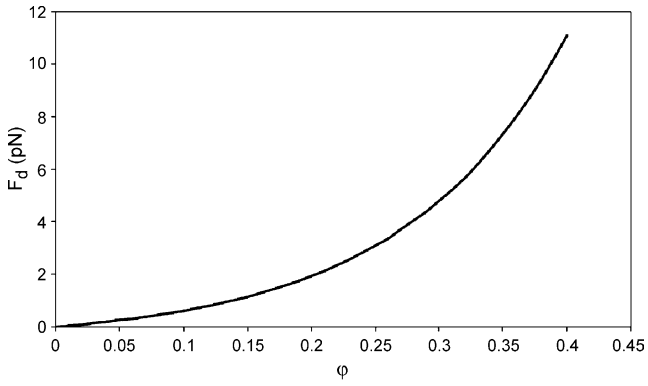

Fig. 6. Maximum depletion force (F_d) versus the volume fraction of crowders (ϕ) of radius 3 nm. F_d is in unit of pN.

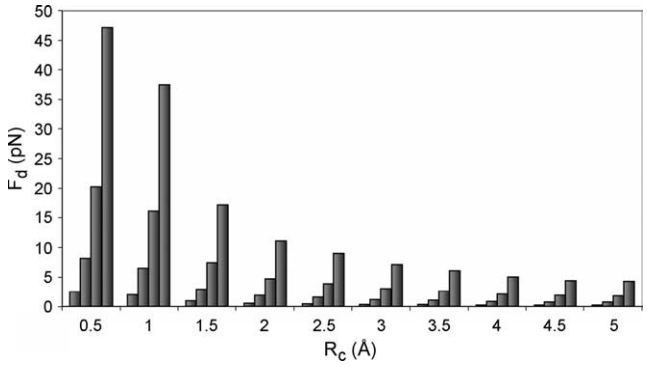

Fig. 7. The maximum depletion force plotted as a function of both the crowder volume fraction and crowder size. Each number along the abscissa is the crowder size in unit of 1 nm. Each size group includes four increasing crowder volume fractions: 10, 20, 30, and 40%, respectively.

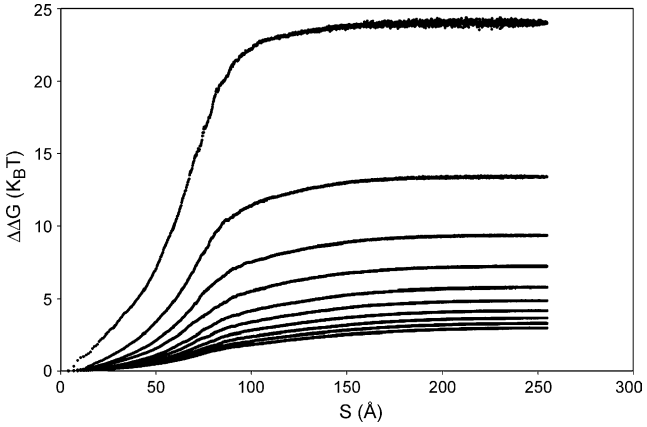

Fig. 8. Depletion free energy (in unit of k_BT) plotted against end-to-end distance (in unit of 0.1 nm) of the polymer at a fixed volume fraction of 28%. From the top down the crowder size goes from 0.5 to 5 nm uniformly with 0.5 nm spacing.

50 nm/s pulling rate. However, the experimental value may decrease, if the limit of zero pulling rate is taken. The above theoretical calculation is done at this limit.

Related to some experimental measurements, e.g. Ref. [4], it is interesting to investigate how depletion force varies with the crowder size, when the volume fraction of the crowders is fixed. In Fig. 7, depletion force is plotted as a function of crowder radius and volume fraction. The figure shows that the depletion force decreases rapidly with R_c for a fixed φ. The main contribution seems to come from the number density factor in the osmotic expression, Eq. (6), where φ is fixed. A minor contribution may come from the derivative factor, $\partial V_{ex}/\partial a$, in Eq. (4). Thus depletion force is essentially inversely proportional to R_c.

By integrating the depletion force over the reaction coordinate for protein folding, here the end-to-end distance, one can obtain $\Delta\Delta G = \Delta(G_u - G_f) = (G_u - G_f)_c - (G_u - G_f)_0$. This is a measure of the change of free energy (or stability) between the folded conformation (f) and unfolded conformation (u) due to the effect of macromolecular crowding, denoted by the subscript c. Integrating Eq. (4), one obtains,

$$\Delta\Delta G(S) = \int_{S_{min}}^{S} F_d(s)\mathrm{d}S = p_{os}[V_{ex}(s) - V_{ex,min}], \quad (7)$$

where $V_{ex}(S)$ and $V_{ex,min}$ are the excluded volumes at the end-to-end distance S and at the minimal S, S_{min}. When S equals to the average S for the ensemble of unfolded conformations, $\Delta\Delta G(S)$ becomes equal to $\Delta\Delta G$, as defined above. Examples of $\Delta\Delta G(S)$ are shown in Fig. 8, which shows that $\Delta\Delta G(S)$ increases with S and overall change of free energy, $\Delta\Delta G$, decreases with the crowder size for a fixed volume fraction. Our results seem to be in the right order of magnitude (e.g. $\Delta\Delta G = 4.2 k_B T$ for $R_c = 3$ nm) when compared to other studies [19].

6. Discussion and conclusion

Based on the concept of depletion force we have calculated the enhancement of the mechanical force required to unfold an ubiquitin molecule in a solution of dextran. Our calculations are done using an off-lattice 3D polymer model and the change of the excluded volume as protein unfolds is calculated. Using this quantity and an expression for the osmotic pressure of dextran from the scaled particle theory, we calculated the depletion force due to macromolecular crowding. Our theoretical results are only in qualitative agreement with our experimental measurements, carried out in single-molecule AFM stretching experiments on ubiquitin.

We discuss some of possible sources of discrepancy in this section. The theoretical calculations and simulations are carried out for systems in equilibrium, while the mechanical force-induced unfolding of protein molecules observed in our experimental measurements is a process far from equilibrium, even at the slowest pulling speed used in the experiments (50 nm/s). As usual, the maximal unfolding force increases with the pulling rate due to dissipated energy [39]. Therefore, the predicted value for the unfolding force represents a lower limit of the measured force.

The discrepancy could also partly be due to the fact that the surface area of a real protein and thus its excluded volume are very different from what are calculated here based on a 3D bead model, where the surface area is relatively smooth compared to a real protein, which has more complicated backbone and side chain structures. It may be that our polymer model is more suitable for simulations of random coil-compact transitions of homopolymers than folding transitions of real proteins. We are here using the former transitions to approximate the latter transitions.

Continuing along the same reasoning, it is believed that the formalism derived in the present article based on the theory of depletion force and scaled particle theory is essentially correct, but the method of calculating the excluded volume can be improved. Therefore, a next step could be static calculations using an all-atom models for a protein, based on which we should calculate the surface area and excluded volume of real protein in its native and mechanically stretched conformations.

Other possibilities related to measurements which at this point still need to be eliminated are that specific interactions exist between dextran molecules and ubiquitin which stabilize the native conformation more relative to the unfolded conformations. The effects of pulling rate and solution viscosity may change the magnitude and the shape of functional relation between the extra mechanical force required and crowder concentration. Related to these possibilities, more experiments using different crowders will be carried out.

Acknowledgements

We thank Dr Chia-Lin Chyan for preparing the unbiquitin polymers. This work was supported in part by National Institute of Health (GM071793) to GY, and a Synergy Grant administered by Drexel University to JMY.

References

[1] Laurent TC. Biochem J 1963;89:253.
[2] Laurent TC, Ogston A. Biochem J 1963;89:249.
[3] Minton AP, Wilf J. Biochemistry 1981;20:4821.
[4] Hall D, Minton AP. Biochim Biophys Acta 2003;1649:127.
[5] Ellis RJ. Trends Biochem Sci 2001;26:597.
[6] Ellis RJ, Minton AP. Nature 2003;425:27.
[7] Sasahara K, McPhie P, Minton AP. J Mol Biol 2003;326:1227.
[8] Yang G, Chyan CL, Ping G, Yuan JM. Submitted for publication.
[9] Asakura S, Oosawa F. J Polym Sci 1958;32:183.
[10] Vrij A. Pure Appl Chem 1976;48:471.
[11] Dinsmore AD, Wong DT, Nelson P, Yodh AG. Phys Rev Lett 1998;(80):409.
[12] Kulkarni AM, Chatterjee AP, Schweizer KS, Zukoski F. Phys Rev Lett 1999;(83):4554.
[13] Verma R, Crocker JC, Lubensky TC, Yodh AG. Phys Rev Lett 1998;(81):4004.
[14] Minton AP. Biopolymer 1981;20:2093.
[15] Minton AP. J Biol Chem 2001;276:10577.
[16] Minton AP. Biophys J 2000;78:101.
[17] Zhou HX. J Mol Recognit 2004;17:368.
[18] Zhou HX, Dill KA. Biochemistry 2001;40:11289.
[19] Minton AP. Biophys J 2005;88:971.
[20] Ping G, Yuan JM, Vallieres M, Sun Z, Wei Y, Dong H, et al. J Chem Phys 2003;118:8042.
[21] Ping G, Yuan JM, Sun Z, Wei Y. J Mol Recognit 2004;17:433.
[22] Ping G. PhD Dissertation, Drexel University; 2005.
[23] Chan HS, Dill KA. J Chem Phys 1994;100:9238.
[24] Friedel M, Sheeler DJ, Shea JE. J Chem Phys 2003;118:8106.
[25] Klimov D, Newfield D, Thirumalai D. PNAS 2002;99:8019.
[26] Cheung MS, Klimov D, Thirumalai D. PNAS 2005;102:4753.
[27] Harries D, Parsegian VA. Proteins 2004;57:311.
[28] Kinjo AR, Tanaka S. Phys Rev A 2002;66(31911):51902.
[29] Yang G, Cecconi C, Baase W, Vetter I, Breyer W, Haack J. Proc Natl Acad Sci, USA 2000;97:139.
[30] Chyan CL, Lin FC, Peng H, Yuan JM, Chang CH, Lin SH, et al. Biophys J 2004;87:3995.
[31] Weiss M, Elsner M, Kartberg F, Nilsson T. Biophys J 2004;87:3518.
[32] Carrion-Vazquez M, Li H, Lu H, Marszalek PE, Oberhauser AF, Fernandez JM. Nat Struct Biol 2004;10:738.
[33] Fernandez JM, Li H. Science 2004;303:1674.
[34] Lebowitz JL, Helfand E, Praestgaard E. J Chem Phys 1965;43:774.
[35] Carnahan NF, Starling KE. Phys Rev A 1970;1:1672.
[36] Reiss H. Adv Chem Phys 1965;9:1.
[37] Kennedy T. J Stat Phys 2002;106:407.
[38] Hsu HP, Mehra V, Nadler W, Grassberger P. J Chem Phys 2003;118:444.
[39] Li FY, Yuan JM, Mou CY. Phys Rev E 2001;63:02195.