Finite Size Effects on Thermal Denaturation of Globular Proteins

Mai Suan Li,[1,2] Dmitri K. Klimov,[3] and D. Thirumalai[2]

[1]*Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46, 02-668 Warsaw, Poland*
[2]*Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742, USA*
[3]*Bioinformatics and Computational Biology Program, School of Computational Sciences, George Mason University, Manassas, Virginia 20110, USA*

(Received 15 September 2003; published 21 December 2004)

Finite size effects on the cooperative thermal denaturation of proteins are considered. A dimensionless measure of cooperativity, Ω_c, scales as N^{ζ}, where N is the number of amino acids. Surprisingly, we find that ζ is universal with $\zeta = 1 + \gamma$, where the exponent γ characterizes the divergence of the susceptibility for a self-avoiding walk. Our lattice model simulations and experimental data are consistent with the theory. Our finding rationalizes the marginal stability of proteins and substantiates the earlier predictions that the efficient folding of two-state proteins requires $T_F \approx T_\theta$, where T_θ and T_F are the collapse and folding transition temperatures, respectively.

DOI: 10.1103/PhysRevLett.93.268107 PACS numbers: 87.10.+e, 36.20.–r, 87.15.Cc

Single domain globular proteins, which are finite-sized systems, undergo remarkably cooperative transitions from an ensemble of unfolded states to well ordered folded (or native) states as the temperature is lowered [Fig. 1(a)]. In many cases, the transition to the native state takes place in an apparent two-state manner; i.e., the only detectable species are the native [more precisely, the conformations belonging to the native basin of attraction (NBA)] or unfolded (U) states [1]. Although the microscopic origin of cooperativity is not fully understood [2], the transition to the NBA at the folding transition temperature, T_F, is a consequence of the effective interresidue attraction that compensates for the entropy loss. From this perspective the NBA $\leftrightarrow$ U transition can be viewed as a phase transition in a finite-sized system. Furthermore, the transition to the NBA at T_F has the characteristics of a weak first order phase transition [1,2]. Many experiments have shown that folded states of globular proteins are only marginally stable below T_F. The free energies of stability of the NBA, relative to the U states, vary within the range of (5–20)k_BT at neutral pH [1(b)]. Because proteins are polymers we expect that they would also undergo a collapse transition to a compact phase at the temperature T_θ suitably modified for finite size systems, when water becomes a poor solvent for the polypeptide. We have previously shown that for protein sequences that fold in an apparent two-state manner $T_F \approx T_\theta$, which naturally explains the marginal stability of proteins [3].

The quest to understand, at the molecular level, the cooperative U $\leftrightarrow$ NBA transition has led to a number of computational studies [2(b),4,5]. Although considerable effort has been directed to describe the molecular basis of cooperativity, somewhat surprisingly, examination of the finite size effects in the self-assembly of proteins has received little attention [6]. In contrast, scaling theories for finite-sized systems undergoing regular first and second order phase transitions have been fully developed [7]. The purpose of this Letter is to study the effect of N, the number of amino acid residues in a protein, on the extent of cooperativity in the U $\leftrightarrow$ NBA transition.

We use thermal denaturation data for wild-type (WT) proteins and LMs of polypeptide chains to examine the dependence of the cooperativity on N. We show that a dimensionless measure of cooperativity [5]

$$\Omega_c = \frac{T_F^2}{\Delta T} \left| \frac{df_N}{dT} \right|_{T=T_F} \tag{1}$$

grows as

$$\Omega_c \sim N^{\zeta}, \tag{2}$$

where f_N is a measure of occupation of NBA, ΔT is the full width at half maximum of $|df_N/dT|$, and T_F is the folding transition temperature identified with the maximum in df_N/dT. We find that

$$\zeta = 1 + \gamma, \tag{3}$$

where γ is the exponent that characterizes the divergence of susceptibility at the critical point for a n-component ferromagnet with $n = 0$, i.e., for a self-avoiding walk. As a byproduct of this study we also show that $\frac{\Delta T}{T_F} \sim \frac{1}{N}$. The parameter Ω_c is a convolution of the sharpness of the transition ($T_F/\Delta T$) and the extent to which the structure, as measured by f_N, changes around T_F. For infinite systems undergoing sharp transitions, $\Omega_c \to \infty$, whereas Ω_c is small for broad or highly rounded phase transitions [5]. The relationship given in Eq. (3), which can be valid only near T_θ, establishes the proposal that $T_F \approx T_\theta$ for two-state folders [3,8].

To establish the results given above we used thermal and chemical denaturation data together with the LMs of a polypeptide chain to compute the growth of Ω_c with N. In the LM each amino acid is represented as a single bead confined to the vertices of a cubic lattice [2(c)]. The energy of a conformation specified by the positions,

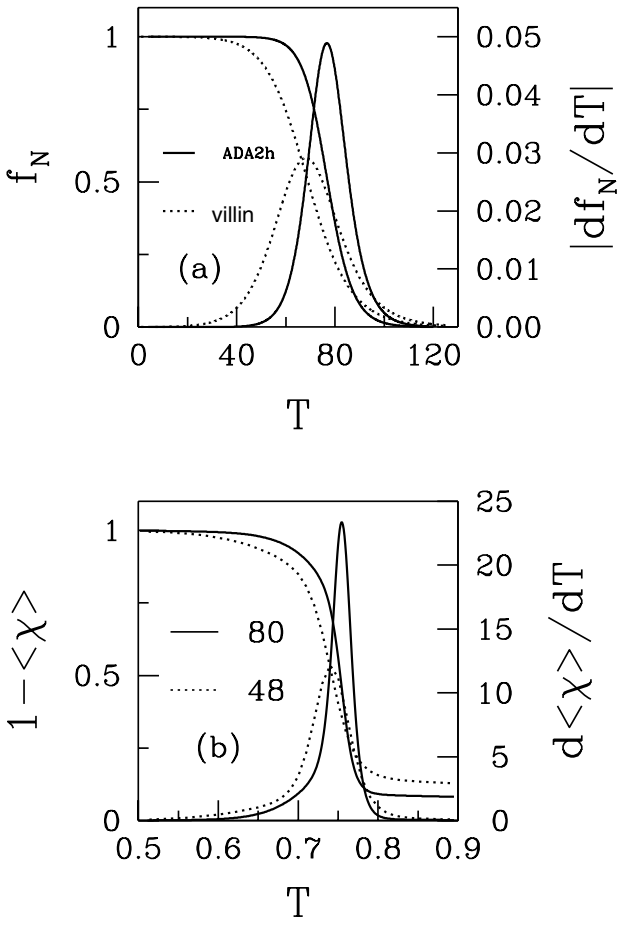

FIG. 1. (a) Temperature dependence of the fraction of occupation of the native state, $f_N(T)$, and its derivative df_N/dT. The dotted lines are for the villin headpiece and the solid lines show the data for ADA2h [12]. Temperature is measured in degrees Centigrade. (b) Dependence of $1-\langle\chi\rangle$ and $d\langle\chi\rangle/dT$ on temperature for lattice models (LMs). We calculate ΔT using $d\langle\chi\rangle/dT$. The dotted lines are for the sequence with $N=48$ and the solid lines correspond to $N=80$.

$\{\vec{r}_i\}$ $(i=1,2,\ldots,N)$, is $E\{\vec{r}_i\}=\sum_{i<j}\epsilon_{ij}\delta_{r_{ij},a}$, where a is the lattice spacing, $r_{ij}=|\vec{r}_i-\vec{r}_j|$, and $\delta_{x,a}$ is the Kronecker delta function. The contact energies $\epsilon_{ij}=-1$, if the interaction between beads i and j in a given conformation is also present in the native state (i.e., the lowest energy conformation for a given sequence), and is zero, otherwise. Even though simple LMs do not quantitatively capture the cooperativity of folding transitions in proteins [9], they are useful for obtaining global folding properties. The precise choice of ϵ_{ij} should not affect the predicted universal scaling of Ω_c with N. Our purpose in undertaking LM Monte Carlo simulations is to show that Eqs. (1)–(3) should be valid for any model of proteins that exhibits a cooperative U $\leftrightarrow$ NBA transition.

To calculate Ω_c for LMs we employ the temperature dependence of the overlap function [3]

$$\chi=1-\frac{1}{N^2-3N+2}\sum_{i<j+1}^{N}\delta_{r_{ij},r_{ij}^0}, \tag{4}$$

where r_{ij}^0 is the distance between beads i and j in the native conformation. The overlap function χ is an order parameter that distinguishes the NBA and U states. The folding transition temperature T_F can be estimated from the location of the maximum in $d\langle\chi\rangle/dT$, where $\langle\cdots\rangle$ indicates a thermal average. For LMs $\langle\chi\rangle\approx 1-f_N$ [10]. Therefore, Eq. (1) may be evaluated using

$$\Omega_c=\frac{T_F^2}{\Delta T}\left(\frac{d\langle\chi\rangle}{dT}\right)_{T=T_F}. \tag{5}$$

Analysis of experimental and simulation data.—To establish the results given above we first analyzed thermal denaturation data for WT proteins. As an example we show in Fig. 1 the plots of $f_N(T)$ and $df_N(T)/dT$ for villin ($N=35$) and ADA2h ($N=80$) [12]. In accord with Eq. (2) we find that the thermal denaturation of ADA2h is more cooperative than that of the villin headpiece.

From Fig. 2 we find that $\frac{\Delta T}{T_F}$, from thermal denaturation data for 32 WT proteins [12], scales as $N^{-\lambda}$ with $\lambda=1.08\pm0.04$. Given that the data for these proteins are obtained under varying experimental conditions and using different methods for computing the enthalpy and entropy changes at T_F, the agreement between the predicted and observed behavior is excellent. For LMs $\Delta T/T_F\sim N^{-\lambda}$

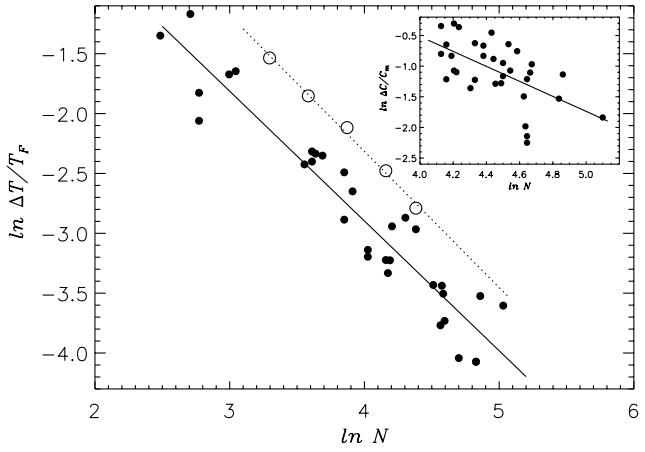

FIG. 2. The sharpness of the folding transition $\Delta T/T_F$ as a function of N. Open circles represent the results from LM simulations with the corresponding fit (dotted line) $\Delta T/T_F\sim N^{-\lambda}$ with $\lambda=1.14\pm0.08$. The linear fit (solid line) to the experimental data for 32 WT proteins (solid circles) [12] gives $\lambda=1.08\pm0.04$. The correlation coefficient for WT $\ln\Delta T/T_F$ and $\ln N$ is 0.95. For clarity LM data are shifted up by 0.4. The inset shows the dependence of the width of the folding transition $\ln\Delta C/C_m$ for chemical denaturation on $\ln N$. The linear fit to the data points collected for 33 WT proteins yields $\lambda=1.22\pm0.14$ (the correlation factor is 0.59).

with $\lambda = 1.14 \pm 0.06$ (Fig. 2). The small deviation of λ from unity in LMs is, in all likelihood, due to the simplicity of the α-carbon representation of the polypeptide chain that does not capture the crucial role of side chains. The inclusion of side chains, which are tightly packed in native conformations, is expected to reduce fluctuations. Moreover, for $N \lesssim 40$ most of the beads are on the surface, which also leads to considerable conformational fluctuations. Therefore, the expected relation $\frac{\Delta T}{T_F} \sim N^{-1}$ holds nearly quantitatively.

The dependence of Ω_c on N for WT proteins and LMs shows that $\Omega_c \sim N^{\zeta}$ (Fig. 3). From the linear fit to the log-log plot of the data we find $\zeta \approx 2.17 \pm 0.09$ for WT proteins and $\approx 2.33 \pm 0.08$ for LMs. The fifth order ϵ expansion for polymers using n-component ϕ^4 theory with $n = 0$ gives $\gamma = 1.22$ [13]. Thus, from Eq. (3) we predict that $\zeta \approx 2.22$. Thus, the data for WT proteins and LMs are consistent with the theoretical prediction [Eq. (3)]. We should emphasize that the robustness of the fit has been checked using different fitting procedures. The remarkable finding relating the critical exponent γ to thermal denaturation of proteins gives further credence to the proposal that efficient folding is achieved at $T_F \approx T_\theta$ [3]. It also suggests that U $\leftrightarrow$ NBA transition is only weakly first order, thus explaining the marginal stability of globular proteins.

Most folding experiments are performed by titrating with denaturants (urea or guanidine hydrochloride). At denaturant concentrations above the midpoint C_m (at which the populations of the folded and unfolded states are equal) proteins are denaturated. Thus, phase transitions to the NBA occur by varying denaturant concentration. In analogy with Eq. (1) we computed, for 33 WT proteins [12], $\Omega_c = \frac{C_m^2}{\Delta C} |\frac{df_N}{dC}|_{C=C_m}$ and $\frac{\Delta C}{C_m}$, where ΔC is the full width at half maximum of $|df_N/dC|$. The plots of $\ln\frac{\Delta C}{C_m}$ and $\ln\Omega_c$ as a function of $\ln N$ yield $\lambda \approx 1.22 \pm 0.14$ and $\zeta \approx 2.45 \pm 0.29$, respectively (see the insets to Figs. 2 and 3). Thus, the scaling of Ω_c and $\Delta C/C_m$ remains essentially unchanged even though the chemical and thermal denaturation mechanisms are vastly different. This result also suggests that ζ is universal. However, the dependence of $\ln\Omega_c$ on $\ln N$ has a correlation coefficient of only about 0.6 compared to 0.95 for thermal denaturation. We believe that larger uncertainties result from a shorter span of N and greater drift in the experimental signals in denaturant-induced unfolding compared to thermal denaturation [3].

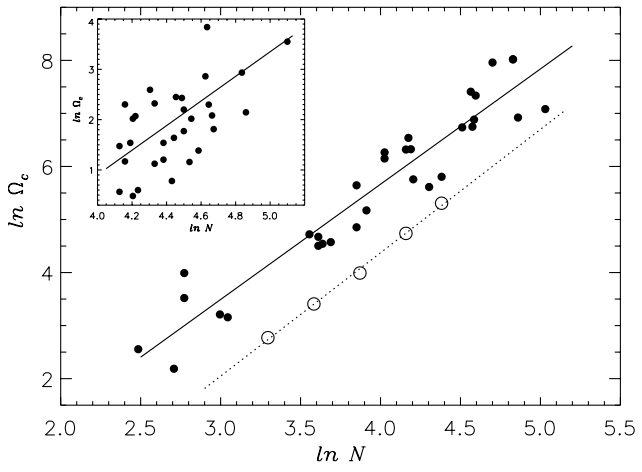

FIG. 3. Plot of $\ln\Omega_c$ as a function of $\ln N$. Symbols are the same as in Fig. 2. The dotted line is a fit to the LM data, which gives $\zeta = 2.33 \pm 0.08$. The solid line is a fit to the experimental values of Ω_c [12] with the exponent $\zeta = 2.17 \pm 0.09$. The correlation coefficient for WT $\ln\Omega_c$ and $\ln N$ is 0.95. The LM data are shifted down by 0.7. The dependence of the folding cooperativity on N for chemical denaturation is plotted in the inset. The linear fit to experimental data (solid line) results in the exponent $\zeta = 2.45 \pm 0.29$ (the correlation factor is 0.59). Both sets of experimental data rule out $\zeta = 2$.

The rationale for Eqs. (2) and (3) is based on the following arguments. (i) By analogy with magnetic systems $\Delta\chi$ is similar to susceptibility and should be given by $\Delta\chi = T\partial\langle\chi\rangle/\partial h$, where h is a "magnetic" or an ordering field conjugate to χ. Because $\Delta\chi$ is dimensionless, we expect that the ordering field $h \sim T$ and thus $Td\langle\chi\rangle/dT$ in proteins is similar to magnetic susceptibility. (ii) Camacho and Thirumalai [3] have suggested that efficient folding in apparent two-state folders requires $T_F \approx T_\theta$. Because the transition at T_θ is usually second order [14], while the one at T_F is first order [2(c),14], the $T_F \approx T_\theta$ condition implies that folding of two-state globular proteins occurs near a tricritical point [3]. Therefore, the critical exponents that control the behavior of the polypeptide chain at T_θ should manifest itself in the U $\leftrightarrow$ NBA phase transition. Using these arguments we can obtain the N dependence of Ω_c in the following way. In general, we expect that close to $T \approx T_\theta \approx T_F$ the Flory radius [15] $R_F \sim \Delta T^{-\nu} \sim N^{\nu}$ (R_F is the analog of the correlation length in magnetic systems). This implies that $\Delta T/T_F \sim N^{-1}$. Because of the analogy to magnetic susceptibility, we expect $Td\langle\chi\rangle/dT \sim N^{\gamma}$. Using Eq. (5) we obtain the expected relationship $\Omega_c \sim N^{1+\gamma}$, which directly follows from the hypothesis that $T_F \approx T_\theta$ for efficient two-state folders [3].

The scaling $\Omega_c \sim N^{\zeta}$ with ζ clearly different from two may appear to be at odds with the idea that the structures in the NBA are sequence specific. However, the global characteristics embodied in the growth of Ω_c with N are valid only at $T \approx T_F$. In the neighborhood of this temperature the general characteristics of the U $\leftrightarrow$ NBA transition are governed by the properties of the unfolded states as T_F is approached from above. It has been shown that in the denaturated states ($T > T_F$) the global properties like the gyration radius $R_g \sim N^{\nu}$ with $\nu \approx 0.59$ as expected for homopolymers [16]. Similarly, the homopolymeric nature around T_F is reflected in the growth of Ω_c with N.

The finding that the folding transition at T_F occurs at a tricritical point suggests that the native states of natural proteins are only marginally stable. Because biological functions require transitions between different states, it is logical to postulate that natural foldable proteins have evolved to ensure $T_\theta \approx T_F$. The coil-globular transition at T_θ is likely to be a second order transition involving no discontinuity in the free energy. At T_F the transition is of the first order. The closeness of T_F and T_θ implies that the discontinuity of the free energy at T_F cannot be large. As a result the folded state is expected to be only marginally stable with respect to the ensemble of denatured states. As argued elsewhere [8] this condition is also equivalent to maximizing the ratio T_F/T_g, where T_g is a glass transition temperature [17]. The marginality condition may also be a requirement for robustness of the folded state. This may explain why small single domain proteins can tolerate a large number of mutations without substantial changes in the native state. It is also likely, as recently shown, that evolution has led to marginally stable proteins that have maximum sequence-structure compatibility [18,19].

This work was supported in part by KBN Grant No. 1P03B01827 and by National Science Foundation Grant No. NSF CHE-0209340. We are grateful to R. B. Dyer, A. R. Fersht, and N. Ferguson for providing us with unpublished thermal denaturation data. M. S. L. is thankful for the hospitality of IPST (USA) and ICTP (Trieste), where part of this work was done.

[1] (a) D. Poland and H. A. Scheraga, *Theory of Helix-Coil Transitions in Biopolymers* (Academic Press, New York, 1970);(b) T. E. Creighton, *Proteins: Structures and Molecular Principles* (W. H. Freeman & Co., New York, 1993); (c) P. L. Privalov, Adv. Phys. Chem. **33**, 167 (1979).

[2] (a) J. D. Bryngelson, J. N. Onuchic, N. D. Socci, and P. G. Wolynes, Proteins: Struct., Funct., Genet. **21**, 167 (1995); (b) A. Kolinski, W. Galazka, and J. Skolnick, Proteins: Struct., Funct., Genet. **26**, 271 (1996); (c) K. A. Dill *et al.*, Protein Science **4**, 561 (1995).

[3] C. J. Camacho and D. Thirumalai, Proc. Natl. Acad. Sci. U.S.A. **90**, 6369 (1993).

[4] M.-H. Hao and H. Scheraga, J. Mol. Biol. **277**, 973 (1998).

[5] D. Klimov and D. Thirumalai, Folding Des. **3**, 127 (1998).

[6] D. K. Klimov and D. Thirumalai, J. Comput. Chem. **23**, 161 (2002); M. S. Li, D. K. Klimov and D. Thirumalai, Polymer **45**, 573 (2004).

[7] (a) M. E. Fisher and M. N. Barber, Phys. Rev. Lett. **28**, 1516 (1972); (b) M. E. Fisher and A. N. Berker, Phys. Rev. B **26**, 2507 (1982).

[8] D. Thirumalai, J. Phys. I (France) **5**, 1457 (1995).

[9] (a) J. Tsai, M. Gerstein, and M. Levitt, Protein Science **6**, 2606 (1997); (b) H. Kaya and H. S. Chan, Proteins: Struct., Funct., Genet. **40**, 637 (2000); (c) H. Kaya and H. S. Chan, J. Mol. Biol. **325**, 911 (2003).

[10] We generated between 12 to 20 distinct maximally compact native structures for a given value of N ($27 \leq N \leq 80$). For example, all native structures for $N = 80$ were confined to the vertices of a $4 \times 4 \times 5$ cube. In the Go model each structure corresponds to a distinct sequence. We used, depending on N, between 50 and 100 Monte Carlo trajectories to collect states and applied the multiple histogram method [11] to compute $\langle \chi(T) \rangle$, $d\langle \chi \rangle / dT$, and Ω_c using Eq. (5). For the Go models (and other highly optimized sequences) T_F, identified with the location of the maximum in $d\langle \chi \rangle / dT$, coincides with T_θ [3].

[11] A. M. Ferrenberg and R. H. Swendsen, Phys. Rev. Lett. **63**, 1195 (1989).

[12] A list of WT proteins together with the values of parameters for thermal and chemical denaturation is available at www.biotheory.umd.edu/ScalingDB.html or binf.gmu.edu/dklimov/Publications/ScalingDB.html. In both datasets the list of proteins spans the whole range of topologies, from peptides with α-helical or β-hairpin structures to all α, all β, or α/β proteins. Because of the spectrum of topologies there is very little sequence similarity. The larger error in ζ and λ for chemical denaturation data is due to the shorter span of N.

[13] H. Kleinert and V. Schulte-Flohlinde, *Critical Properties of ϕ^4-Theories* (World Scientific, Singapore, 2002).

[14] A. Yu. Grosberg and A. R. Khokhlov, *Statistical Physics of Macromolecules* (AIP Press, New York, 1994).

[15] P. G. de Gennes, *Scaling Concepts in Polymer Physics* (Cornell University Press, New York, 1985).

[16] J. E. Kohn *et al.*, Proc. Natl. Acad. Sci. U.S.A. **101**, 12491 (2004).

[17] J. N. Onuchic, Z. A. Luthey-Schulten, and P. G. Wolynes, Annu. Rev. Phys. Chem. **48**, 545 (1997).

[18] Y. Xia and M. Levitt, Proc. Natl. Acad. Sci. U.S.A. **99**, 10382 (2002).

[19] D. M. Taverna and R. Goldstein, J. Mol. Biol. **315**, 49 (2002).