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COMMUNICATION

Native Proteins are Surface-molten Solids: Application of the Lindemann Criterion for the Solid *versus* Liquid State

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Since the internal motions of proteins play an essential role in their biological function, it is important to characterize them in a fundamental way. The Lindemann criterion for the solid state is applied to molecular dynamics simulations and temperature-dependent X-ray diffraction data of proteins. It is found that the interior of native proteins is solid-like, while their surface is liquid-like. When the entire protein becomes solid-like at low temperature (~220 K), the protein is inactive. Thus, the surface-molten solid nature of proteins in their native state permits the dynamics required for function, while preserving their stability. Comparison with rare gas clusters and polymer models indicates that their thermodynamic phase diagrams have many elements in common with those of proteins.

Keywords: protein thermodynamics; glass transition; internal fluctuations; protein dynamics; phase diagram

Native proteins were described as aperiodic crystals by Schrödinger (1944). In his view, proteins were solids but did not have the periodic regularity of ordinary crystals. Structural data have, in fact, shown that the densities of proteins are more like hydrocarbon solids than liquids (Richards & Lim, 1993) and thermodynamic analyses indicate that the non-polar interactions are comparable to those of solid hydrocarbons on average (Lazaridis *et al.*, 1995; Makhatadze & Privalov, 1995). These results can be contrasted with molecular dynamics simulations (McCammon *et al.*, 1977; Elber & Karplus, 1987; Kneller & Smith, 1994; Brooks *et al.*, 1988) and experimental measurements (Alberding *et al.*, 1976; Artymiuk *et al.*, 1979; Wüthrich & Wagner, 1978; Kossiakoff & Shteyn, 1984), which suggest that the internal motions of proteins have liquid-like characteristics. From a study of myoglobin based on an X-ray and Mössbauer data for the atomic mean-square fluctuation, Frauenfelder *et al.* (1979) suggested that a protein had an aperiodic solid core with ''semi-liquid''-like outside (see also Frauenfelder & McMahon, 1998). This is consistent with the fact that the packing on the surface of proteins is looser than in the protein interior (Gerstein & Chothia, 1996). Here, we make use of the Lindemann criterion (Lindemann, 1910) to characterize the internal dynamics of proteins. The Lindemann criterion was introduced for determining whether an infinite system is solid-like or liquid-like (Bilgram, 1987; Löwen, 1994). It has been used also for finite systems, such as Lennard-Jones clusters (Stillinger & Stillinger, 1990), isolated homopolymers (Zhou *et al.*, 1997) and protein-like heteropolymers (Zhou & Karplus, 1997). In terms of the Lindemann criterion, studies of the experimental temperature factors and molecular dynamics simulations show that proteins are surface-molten solids at physiological temperatures, consistent with the results obtained from a simplified protein model (Zhou & Karplus, 1997). Further, the analysis indicates that the so-called glass transition in proteins (Green *et al.*, 1994) occurs at a temperature where the molecule becomes solid-like. This is likely to be the cause of the fact that certain proteins have been shown to become inactive at the ''glass'' transition temperature (Tilton *et al.*, 1992; Ferrand *et al.*, 1993; Reat *et al.*, 1998).

The Lindemann criterion is related to a ''disorder'' parameter that was introduced in 1910 (Lindemann, 1910). It has been applied to study

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solid *versus* liquid behavior in infinite systems ranging from crystals (Bilgram, 1987) such as rare-gas solids, metals and alkali halides to model systems (Löwen, 1994) such as hard spheres and Yukawa spheres. The original Lindemann criterion compares the atomic fluctuation amplitude $\langle \Delta r^2 \rangle^{1/2}$ with the lattice constant a of a crystal. If this ratio, which is defined as the disorder parameter, Δ_L, reaches a certain value, fluctuations cannot increase without damaging or destroying the crystal lattice. The results of experiments and simulations show that the critical value of Δ_L for simple solids is in the range of 0.1 to 0.15, relatively independent of the types of substance, the nature of the interaction potential, and the crystal structure (Bilgram, 1987; Löwen, 1994; Stillinger, 1995). Application of this criterion to an inhomogeneous finite system like a protein requires evaluation of the generalized Lindemann parameter (Stillinger & Stillinger, 1990):

$$\Delta_L = \frac{\sqrt{\Sigma_i \langle \Delta r_i^2 \rangle / N}}{a'} \tag{1a}$$

$$\Delta_L^{in}(r_{\rm cut}) = \frac{\sqrt{\Sigma_{i, r_i < l t r_{\rm cut}} \langle \Delta r_i^2 \rangle / N}}{a'} \tag{1b}$$

where N is the number of atoms and a' is the most-probable non-bonded near-neighbor distance, $\mathbf{r}_i$ is the position of atom i, $\Delta r_i^2 = (\mathbf{r}_i - \langle \mathbf{r}_i \rangle)^2$, and $\langle\rangle$ denotes configurational averages. The dynamics as a function of the distance from the geometric center of the protein is characterized by defining $\Delta_L^{in}(r_{\rm cut})$, which is obtained by averaging over the atoms that are within a chosen cutoff distance, $r_{\rm cut}$, from the center.

One of the key quantities in the Lindemann parameter is the root-mean-squared fluctuation. Here, both experimental and theoretical methods are used for estimating the fluctuations of four proteins (ribonuclease A, barnase, myoglobin and crambin). For ribonuclease A, the fluctuations are determined from the X-ray temperature factors (Tilton *et al.*, 1992), while they are calculated for the three other proteins from molecular dynamics trajectories (after removing the overall translation and rotation of the protein). The barnase trajectory is a 260 ps stochastic boundary molecular dynamics simulation for the native state at 300 K in an explicit water model for the solvent (Caflisch & Karplus, 1995). The polar-hydrogen parameter set 19 (Neria *et al.*, 1996) and the CHARMM program (Brooks *et al.*, 1983) were used; simulation details are described by Calflisch & Karplus (1995). The myoglobin trajectory at 300 K is obtained from a simulation of myoglobin enclosed in a first-layer hydration shell using the CHARMM program (Brooks *et al.*, 1983) with parameter set 22 (MacKerell *et al.*, 1998) at 300 K for 300 ps; simulation details are described by Vitkup *et al.* (1997). The crambin results are based on a 200 ps molecular dynamics vacuum trajectory for the native state; the first 50 ps of the simulation correspond to equilibration and were discarded. The polar hydrogen parameter set 19 (Neria *et al.*, 1996) and the CHARMM program (Brooks *et al.*, 1983) were used; a distance-dependent dielectric constant was introduced in the absence of explicit solvent. Use of these varied models for the calculations demonstrates that the results are not sensitive to the details of the calculational approach.

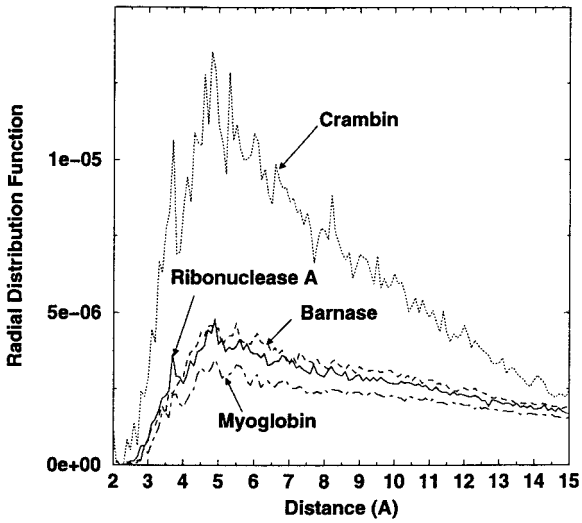

Figure 1. The radial distribution function as a function of distance between two non-bonded heavy-atoms at 300 K for the four proteins considered here.

The other key quantity in evaluating the Lindemann parameter for inhomogeneous systems is the most-probable non-bonded near-neighbor distance, a', in equations (1). To determine a' for the proteins, we plot in Figure 1 the probability of a given heavy-atom non-bonded distance as a function of that distance at 300 K for the four proteins considered here; the values are calculated from average structures obtained from molecular dynamics simulations of crambin, barnase and myoglobin, and from the crystal structure for ribonuclease A (Tilton *et al.*, 1992). A pair of non-bonded atoms is defined to include all heavy-atoms except those in the same residue and the main-chain atoms of neighboring residues. Since the maximum of the distribution is between 4 and 5 Å, we choose the value 4.5 Å for a' to use with equations (1). The distributions at different temperatures for crambin (from simulated average structures) and for ribonuclease A (from the crystal structures; Tilton *et al.*, 1992) are nearly identical with those shown in Figure 1. Similar distributions are obtained when only heavy-atoms within a cut-off distance from the center are used in calculations; the slightly looser packing outside of $r_{\rm cut} = 6$ Å yields the most probable value $a' \sim 4.7$ Å, which has no effect on the considerations developed here. Thus, the parameter a' is relatively insensitive to temperature variation and to the distance away from the protein center.

Table 1. The heavy-atom $\Delta_L(\Delta_L^{in})$ values for four proteins at 300 K

	$\Delta_L(\Delta_L^{in}(6\ \text{Å}))$ MD simulations			X-ray data
Proteins	Barnase	Myoglobin	Crambin	Ribonuclease A
All atoms	0.21 (0.12)	0.16 (0.11)	0.16 (0.09)	0.16 (0.12)
Backbone atoms only	0.16 (0.10)	0.12 (0.09)	0.12 (0.08)	0.13 (0.10)
Side-chain atoms only	0.25 (0.14)	0.18 (0.12)	0.19 (0.10)	0.19 (0.13)

In Table 1, we present the heavy-atom Δ_L and $\Delta_L^{in}(r_{cut})$, with r_{cut} equal to 6 Å, calculated from molecular dynamics trajectories for three proteins and from X-ray temperature factors for ribonuclease A (Tilton *et al*., 1992) at 300 K. The molecular dynamics simulation results for the three proteins based on very different solvation models and those obtained from the experimental temperature factors agree with each other. Although the side-chains show larger fluctuations than the main-chains, as expected (McCammon *et al*., 1977; Elber & Karplus, 1987; Kneller & Smith, 1994; Brooks *et al*., 1988; Alberding *et al*., 1976; Frauenfelder *et al*., 1979; Wüthrich & Wagner, 1978; Kossiakoff & Shteyn, 1984), the same distinction between Δ_L and $\Delta_L^{in}(r_{cut})$ is evident. For convenience, the cutoff radius r_{cut} for $\Delta_L^{in}(r_{cut})$ is taken to be 6 Å for all proteins. Different cutoff distances would lead to slightly different $\Delta_L(r_{cut})$ values for the core but the qualitative picture would remain the same.

The determination of solid-like or liquid-like behavior requires an estimate for the critical value of the Lindemann disorder parameter for proteins. It is expected to be in the range 0.1 to 0.15 found for other systems (Bilgram, 1987; Löwen, 1994; Stillinger, 1995). To assess the critical value, we plot in Figure 2 the temperature-dependence of Δ_L and Δ_L^{in} for crambin obtained by molecular dynamics simulations and compare it with the result for ribonuclease A obtained by X-ray diffraction studies (Tilton *et al*., 1992). The simulation results for crambin show behavior very similar to that for ribonuclease A. In both cases, Δ_L and Δ_L^{in} increase with temperature and there appears to be a dynamic transition around 220 K; the results for crambin also suggest a transition around 330 K, associated with the increased fluctuations expected on denaturation. The transition at 220 K corresponds to a critical value of 0.14 for Δ_L. The transition at 330 K for crambin appears to have a critical value of 0.14 for Δ_L^{in}, suggesting that the protein core becomes liquid-like at this temperature. A similar critical value is found to be appropriate for the calculated root-mean-squared fluctuations of carbon monoxymyoglobin at the dynamic transition temperature (Steinbach & Brooks, 1994). Also, recent simulations of an isolated homopolymer of freely jointed 64 square-well beads has a critical value of 0.12 for the liquid-to-solid transition of the interior (Zhou *et al*., 1997) and a cluster of 55 LJ atoms has a critical value of about 0.15 (Stillinger & Stillinger, 1990).

The present results show that based on the Lindemann criterion for distinguishing solid-like and liquid-like behavior, the interior of a protein ($\Delta_L^{in} < 0.14$) is solid-like, while its surface is liquid-like ($\Delta_L > 0.14$) under physiological conditions. The beginning of thermal denaturation in the simulations appears to be related to the melting of its interior (i.e. $\Delta_L^{in} > 0.14$), so that the entire protein becomes liquid-like. These results are in accord with the detailed phase diagram obtained from the simulation of a three-helix bundle protein represented by the freely jointed square-well model (Zhou & Karplus, 1997). The surface-molten solid of proteins is likely to be essential for protein stability and function. The existence of a solid-like core

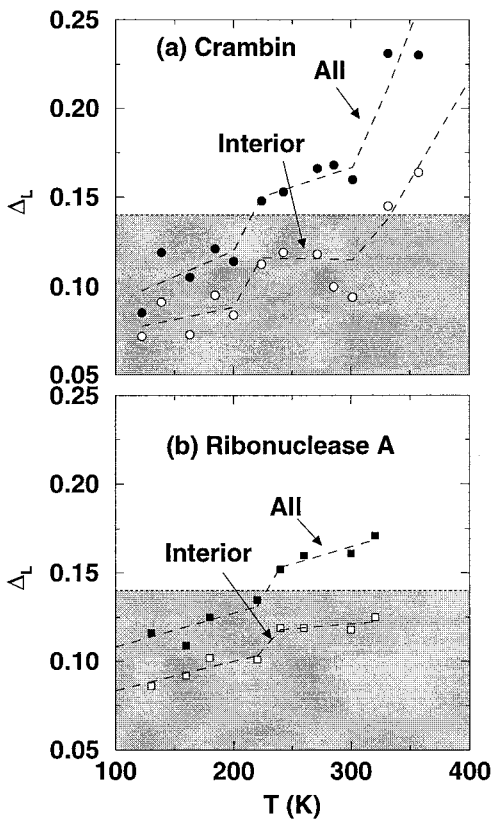

Figure 2. The Lindemann disorder parameter as a function of temperature (K). The connecting lines serve only as a visual guide. The transition between solid and liquid is taken to be 0.14 and is shown as shadow for solid and blank for liquid. The results for (a) crambin are obtained from 200 ps simulations and the results for (b) ribonuclease A are calculated from the X-ray temperature factors.

in native proteins provides a basis for the cooperative transition from compact globules to the native state (Ptitsyn, 1995), in analogy with the liquid-to-solid transition of the interior of compact globules found in homopolymer (Zhou *et al*., 1997) and protein-like heteropolymer (Zhou & Karplus, 1997). It is also in accord with the importance of the conservation of core residues and the similarity of the core structure in proteins with closely related sequences (Chothia & Lesk, 1986; Chothia *et al*., 1998). The cooperativity of the liquid-to-solid-like folding transition is necessary to ensure that the native state be stable and that partially denatured, inactive, structures have negligible probability at ambient temperatures (Karplus & Shakhnovich, 1992). On the other hand, liquid-like motions in surface regions are necessary for activity, as illustrated by the fact that ribonuclease A (Rasmussen *et al*., 1992) and bacteriorhodopsin (Ferrand *et al*., 1993; Reat *et al*., 1998) become inactive below the glass transition to the solid state of the entire protein.

The Lindemann criterion may be of interest in interpreting data on the flexibility and stability in proteins that have evolved to function at different temperatures. It has been pointed out recently, in accord with earlier suggestions (Jaenicke & Zavodszky, 1990), that for the cold adaption, orthologs of the enzyme lactate dehydrogenase A_4 in Antarctic fish increase the flexibility of the structures surrounding the active site (Fields & Somero, 1998).On the other hand, *Thermus thermophilus* IPMDH, which is marginally active at room temperature, appears to be significantly more rigid at room temperature than *Escherichia coli* IPMDH (Zavodszky *et al*., 1998). These data are consistent with the conclusion that a surface-molten solid state is required for a protein to be active. However, it should be noted that greater rigidity *per se* would be expected to destabilize a protein entropically (Lazaridis *et al*., 1997) unless it is compensated by an increased enthalpy or a decreased entropy of unfolding. The latter appears to be achieved in some proteins by a reduction of loop lengths in the thermophilic species *versus* its mesophilic counterpart (Macedo-Ribero *et al*., 1996; Sakon *et al*., 1996; Russell *et al*., 1997; M. J. Thompson & D. Eisenberg, personal communication).

The surface-molten solid state described here for polymers and proteins is similar to that observed in rare gas clusters where the particles on the ''melted'' surface in the presence of a frozen core have large-amplitude, highly anharmonic motions and oscillate around a well-defined structure (Berry, 1997). The isomorphism of the phase diagram of proteins, rare gas clusters (Berry, 1997), isolated homopolymers (Zhou *et al*., 1997) and heteropolymers (Zhou & Karplus, 1997) indicates that proteins do not have special thermodynamic states. However, the specific sequences that lead to functionally optimized structures seem also to be required to obtain an unusually stable ground state.

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