Glass Transition in Biomolecules and the Liquid-Liquid Critical Point of Water

Pradeep Kumar,[1] Z. Yan,[1] L. Xu,[1] M. G. Mazza,[1] S. V. Buldyrev,[2,1] S.-H. Chen,[3] S. Sastry,[4] and H. E. Stanley[1]

[1]*Center for Polymer Studies and Department of Physics, Boston University, Boston, Massachusetts 02215, USA*
[2]*Department of Physics, Yeshiva University, 500 West 185th Street, New York, New York 10033, USA*
[3]*Nuclear Science and Engineering Department, Massachusetts Institute of Technology, Cambridge, Massachusetts 02139, USA*
[4]*Jawaharlal Nehru Center for Advanced Scientific Research, Jakur Campus, Bangalore, 560061, India*

(Received 29 May 2006; published 27 October 2006)

Using molecular dynamics simulations, we investigate the relation between the dynamic transitions of biomolecules (lysozyme and DNA) and the dynamic and thermodynamic properties of hydration water. We find that the dynamic transition of the macromolecules, sometimes called a ''protein glass transition,'' occurs at the temperature of dynamic crossover in the diffusivity of hydration water and also coincides with the maxima of the isobaric specific heat C_P and the temperature derivative of the orientational order parameter. We relate these findings to the hypothesis of a liquid-liquid critical point in water. Our simulations are consistent with the possibility that the protein glass transition results from crossing the Widom line, which is defined as the locus of correlation length maxima emanating from the hypothesized second critical point of water.

DOI: 10.1103/PhysRevLett.97.177802 PACS numbers: 61.20.Ja, 61.20.Gy

Both experiments and computer simulation studies have shown that hydrated proteins undergo a ''glasslike'' transition near 200 K [1–5], above which proteins exhibit diffusive motion and below which the proteins are trapped in harmonic modes. An important issue is to determine the effects of hydration water on this dynamical transition [6–10]. Experiments and computer simulations suggested that, when a protein is solvated, the protein glass transition is strongly coupled to the solvent, leading to the question of whether the protein glass transition is directly related to a dynamic transition in the surrounding solvent [11].

It has been hypothesized that the deeply supercooled region of the phase diagram of liquid water may contain a first order phase transition between a high density liquid (HDL) and a low density liquid (LDL) [12]. This line of phase transition has a negative slope in the P-T phase diagram and terminates with a critical point C_2 [12–15], which is located at $T_{C2} = 200 \pm 20$ K and $P_{C2} = 250 \pm 100$ MPa [16]. Upon crossing the first order phase transition line above the critical pressure, the thermodynamic state functions change discontinuously. Below the critical pressure, they rapidly but continuously change upon cooling.

Computer simulations of the five-point transferable intermolecular potential (TIP5P) and Stillinger-Rahman (ST2) potential models show that response functions such as isobaric specific heat C_P and isothermal compressibility have maxima as functions of temperature if the system is cooled isobarically at $P < P_{C2}$ [15]. The loci of these maxima asymptotically approach one another [17] as the critical point is approached, because response functions become expressible in terms of the correlation length which diverges at the critical point. The locus of the correlation length maxima is called the Widom line.

Experimental studies of supercritical water [18] indeed show that various response functions have sharp maxima in the region of the phase diagram above the liquid-vapor critical point C_1, but no direct experimental indication of a liquid-liquid critical point C_2 had been available due to unavoidable crystallization of bulk water. It was found that water remains unfrozen in hydrophilic nanopores for $T >$ 200 K [19,20]. Moreover, when cooled at constant pressure for $P < P_{C2}$, the dynamics changes from non-Arrhenius to Arrhenius at $T = T_{\times}(P)$. The line $T_{\times}(P)$ is located in the range of temperatures between 200–230 K and has a negative slope in the P-T phase diagram. Computer simulations suggest that this line may be associated with $T_W(P)$, the Widom line, near which the local dynamic characteristics must rapidly change from those resembling the properties of HDL at high temperature to those of LDL at low temperature [15].

Here we explore the hypothesis [21] that the observed glass transition in biomolecules is related to the liquid-liquid phase transition using molecular dynamics (MD) simulations. Specifically, we study the dynamic and thermodynamic behavior of lysozyme and DNA in hydration TIP5P water, by means of the software package GROMACS [22] for (i) an orthorhombic form of hen egg-white lysozyme [23] and (ii) a Dickerson dodecamer DNA [24] at constant pressure $P = 1$ atm, several constant temperatures T, and constant number of water molecules N (NPT ensemble) in a simulation box with periodic boundary conditions. We first allow the system to equilibrate at constant temperature and pressure using the Berendsen method. This initial equilibration is followed by a long production run, during which we calculate the dynamic and static properties. Equilibration times vary for different temperatures from a few nanoseconds for high temperatures to as much as 40 ns for low temperatures. The MD for DNA was performed using the Amber force field [25]. For lysozyme simulations, the system consists of a single protein in the native conformation solvated in $N = 1242$

TIP5P water molecules [14,26,27]. These simulation conditions correspond to a ratio of water mass to protein mass of 1.56. The DNA system consists of a single DNA helix with 24 nucleotides solvated in $N = 1488$ TIP5P water molecules, which corresponds to an experimental hydration level of 3.68.

The simulation results for the mean square fluctuations $\langle x^2 \rangle$ of protein are shown in Fig. 1(a). We calculate the mean square fluctuations $\langle x^2 \rangle$ of protein from the equilibrated configurations, first for each atom over 1 ns and then averaged over the total number of atoms in the protein. We find that $\langle x^2 \rangle$ [Fig. 1(a)] changes its functional form below $T_p \approx 242 \pm 10$ K. Moreover, upon cooling, the diffusivity of hydration water exhibits a dynamic crossover from non-Arrhenius [28] to Arrhenius behavior at the crossover temperature $T_\times \approx 245 \pm 10$ K [Fig. 1(c)]. A similar temperature dependence of diffusivity of bulk TIP5P water was observed in Ref. [15], as well as in simulations of silica [29] and a model binary mixture [30], and expected based on the analysis of thermodynamic data for water [31]. The coincidence of $T_\times$ with T_p within the error bars indicates that the behavior of the protein is strongly coupled with the behavior of the surrounding solvent, in agreement with recent experiments [21]. Note that $T_\times$ is much higher than the glass transition temperature estimated for TIP5P as $T_g = 215$ K [14,32]. Thus, this crossover is not likely to be related to the glass transition in water. Here we will explore the possibility that instead it is related to a change in the properties of protein hydration water.

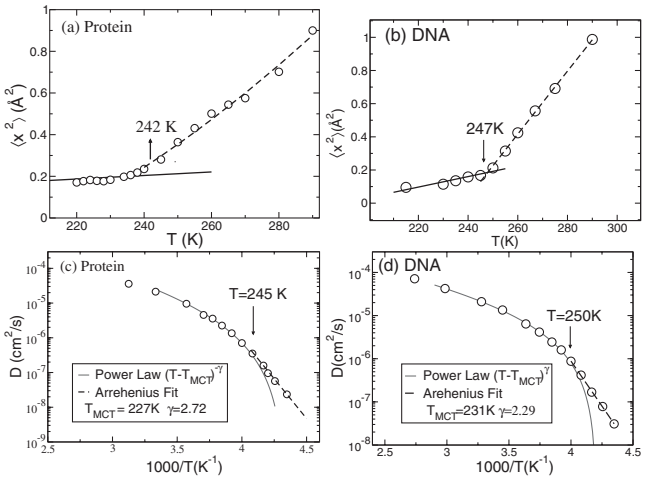

FIG. 1. Mean square fluctuation of (a) lysozyme and (b) DNA showing that there is a transition around $T_p \approx 242 \pm 10$ K for lysozyme and around $T_p \approx 247 \pm 10$ K for DNA. For very low T, one would expect a linear increase of $\langle x^2 \rangle$ with T, as a consequence of harmonic approximation for the motion of residues. At high T, the motion becomes nonharmonic and we fit the data by a polynomial. We determine the dynamic crossover temperature T_p from the crossing of the linear fit for low T and the polynomial fit for high T. We determine the error bars by changing the number of data points in the two fitting ranges. Diffusion constant of hydration water surrounding (c) lysozyme and (d) DNA shows a dynamic transition from a power law behavior to an Arrhenius behavior at $T_\times \approx 245 \pm 10$ K for lysozyme and $T_\times \approx 250 \pm 10$ K for DNA, around the same temperatures where the behavior of $\langle x^2 \rangle$ has a crossover.

We next calculate C_P by numerical differentiation of the total enthalpy of the system (protein and water) by fitting the simulation data for enthalpy with a fifth order polynomial and then taking the derivative with respect to T. Figure 2(a) displays a maximum of $C_P(T)$ at $T_W \approx 250 \pm 10$ K for the case of lysozyme-water system [33]. The fact that $T_p \approx T_\times \approx T_W$ is evidence of the correlation between the changes in protein fluctuations [Fig. 1(a)] and the hydration water thermodynamics [Fig. 2(a)]. Thus, our results are consistent with the possibility that the protein glass transition is related to the Widom line (and hence to the hypothesized liquid-liquid critical point). Crossing the Widom line corresponds to a continuous but rapid transition of the properties of water from those resembling the properties of a local HDL structure for $T > T_W(P)$ to those resembling the properties of a local LDL structure for $T < T_W(P)$ [15,20]. A consequence is the expectation that the fluctuations of the protein residues in predominantly LDL-like water (more ordered and more rigid) just below the Widom line should be smaller than the fluctuations in predominantly HDL-like water (less ordered and less rigid) just above the Widom line.

To test this interpretation, we analyze the structure of hydration water on the two sides of the Widom line. Figure 3(a) shows the oxygen-oxygen radial distribution function $g(r)$ on two sides of the Widom line for lysozyme hydration water. The first peak of $g(r)$ on the low temperature ($T = 230$ K) side is sharper, and the first minimum is shallower compared to the $g(r)$ on the high temperature ($T = 270, 300$ K) side of the Widom line, suggesting that water is more structured on the low temperature side. Further, we calculate the structure factor $S(q)$ of lysozyme hydration water [Fig. 3(c)]. The first peak of the structure factor associated with the hydrogen bond is very sharp and pronounced for $T < T_W(P)$, and it is diminished and moves to larger wave vectors for $T > T_W(P)$, consistent with a LDL-like local structure for $T < T_W(P)$ and a HDL-like local structure for $T > T_W(P)$.

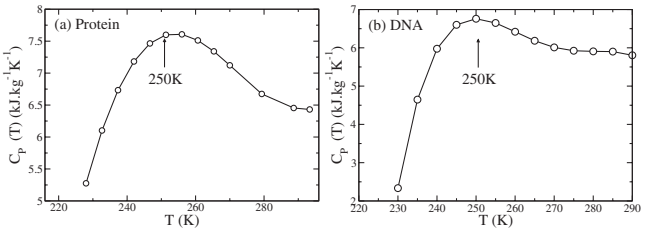

FIG. 2. The specific heat of the combined system (a) lysozyme and water and (b) DNA and water display maxima at 250 ± 10 and 250 ± 12 K, respectively, which are coincident within the error bars with the temperature T_p where the crossover in the behavior of $\langle x^2 \rangle$ is observed.

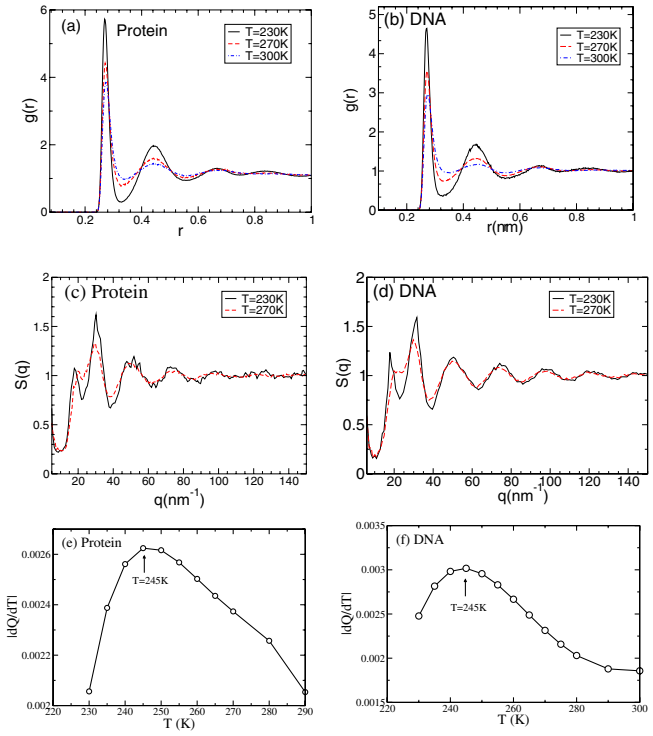

FIG. 3 (color online). Oxygen-oxygen pair correlation function $g(r)$ for (a) lysozyme hydration water and (b) DNA hydration water, on crossing the Widom line from the HDL side ($T = 270, 300$ K) to the LDL side ($T = 230$ K). Structure factor of hydration water surrounding (c) lysozyme and (d) DNA on two sides of the the Widom line. Upon crossing the Widom line, the local structure of water changes from more HDL-like to more LDL-like, reflected in the sharper and more prominent first peak. The first peak associated with the hydrogen bond distance also moves to small wave vectors, suggesting a change from the HDL to the LDL-like local structure of water at low temperatures. Derivative with respect to temperature of the local tetrahedral order parameter Q for (e) lysozyme and (f) DNA hydration water. A maximum in $|dQ/dT|$ at Widom line temperature suggests that the rate of change of local tetrahedrality of hydration water has a maximum at the Widom line.

Previous simulations [10] and experiments [9] suggest a ''glasslike'' transition of DNA around temperature 230 K. Hence, to test if the dynamic crossover depends on the solute, we performed a parallel study of the DNA Dickerson dodecamer [24]. We find that fluctuations [34] of the DNA molecule [Fig. 1(b)] change their behavior approximately at the same temperature as lysozyme, with $T \approx 247 \pm 10$ K. The dynamic crossover in the hydration water upon cooling from non-Arrhenius to Arrhenius behavior takes place at $T_\times \approx 250 \pm 10$ K [Fig. 1(d)]. For DNA, hydration water system C_P has a maximum at $T \approx 250 \pm 12$ K, similar to the case of protein [see Fig. 2(b)] [35]. Figures 3(b) and 3(d) show $g(r)$ and $S(q)$ for the DNA hydration water [36]. Further, to describe the quantitative changes in structure of hydration water, we calculate the local tetrahedral order parameter Q [37] for hydration water surrounding lysozyme and DNA. Figures 3(e) and 3(f) show that the rate of increase of Q has a maximum at 245 ± 10 K for both lysozyme and DNA hydration water, respectively; the same temperatures where we find a crossover in the behavior of mean square fluctuations and a change in the behavior of the dynamics of hydration water.

The quantitative agreement of the results for DNA and lysozyme suggests that it is indeed the changes in the properties of hydration water that are responsible for the changes in dynamics of the protein and DNA biomolecules. Our results are in qualitative agreement with recent experiments on hydrated protein [21] and DNA [38] which found the crossover in side-chain fluctuations at $T_p \approx 225$ K.

We thank C. A. Angell, L. Cruz, P. G. Debenedetti, H. Frauenfelder, G. Franzese, L. Liu, F. Mallamace, P. J. Rossky, and B. Urbanc for helpful discussions and NSF for financial support.

Note added.—Recently, we learned of interesting parallel work on silicon, which also interprets structural change in $g(r)$ and $S(q)$ as crossing the Widom line [39]. We also became increasingly aware of the rich literature on the protein glass transition. The views of the experts on the protein glass transition are divided. To date, differing views proposed for the protein glass transition in the literature include: (i) Water plays no key role in the dynamic crossover observed in the protein glass transition [40]; (ii) the protein glass transition is caused by a solvent, but the actual physical mechanism whereby water leads to the protein glass transition is an open question [41–46]; (iii) the protein glass transition is ''driven'' by water's glass transition [47]. However, the dynamic crossover in protein occurs around ~220 K, more than 50% above water's glass transition temperature (≈136 K); (iv) the solvent is a plasticizer that decreases the local viscosity of protein atoms [42]; (v) at low T, $\langle x^2 \rangle$ is dominated by vibrations and, hence, is linear in T. At higher T, the fast β relaxation is sufficiently large to be seen, and, hence, the crossover occurs at T_p [48,49]. Although it has been previously hypothesized that the protein glass transition is due to water, there remains the question of *why*—i.e., what is the mechanism whereby water gives rise to the protein glass transition. We demonstrate by simulations that the dynamic crossover found in biomolecules at about 220 K is directly related to the fact that the surrounding hydration water undergoes thermodynamic, dynamic, and structural changes. Moreover, we identify that these changes in hydration water occur along a path that crosses the Widom line. Thus, we uncover a possible connection between the liquid-liquid phase transition in water and a crossover in both the hydration water dynamics and the protein dynamics.

[1] D. Ringe and G. A. Petsko, Biophys. Chem. **105**, 667 (2003).

[2] H. Hartmann *et al.*, Proc. Natl. Acad. Sci. U.S.A. **79**, 4967 (1982).
[3] A. L. Lee and A. J. Wand, Nature (London) **411**, 501 (2001).
[4] B. F. Rasmussen *et al.*, Nature (London) **357**, 423 (1992).
[5] W. Doster *et al.*, Nature (London) **337**, 754 (1989).
[6] D. Vitkup *et al.*, Nat. Struct. Biol. **7**, 34 (2000).
[7] M. Tarek and D. J. Tobias, Phys. Rev. Lett. **88**, 138101 (2002); Biophys. J. **79**, 3244 (2000).
[8] J. M. Zanotti *et al.*, Biophys. J. **76**, 2390 (1999).
[9] A. P. Sokolov *et al.*, J. Chem. Phys. **110**, 7053 (1999).
[10] J. Norberg and L. Nilsson, Proc. Natl. Acad. Sci. U.S.A. **93**, 10 173 (1996).
[11] A. L. Tournier *et al.*, Biophys. J. **85**, 1871 (2003).
[12] P. H. Poole *et al.*, Nature (London) **360**, 324 (1992).
[13] I. Brovchenko *et al.*, J. Chem. Phys. **118**, 9473 (2003).
[14] I. Brovchenko *et al.*, J. Chem. Phys. **123**, 044515 (2005).
[15] L. Xu *et al.*, Proc. Natl. Acad. Sci. U.S.A. **102**, 16 558 (2005).
[16] The situation is rather complex. Many different scenarios have been proposed in the literature, including a scenario with multiple liquid-liquid critical points [13,14].
[17] P. H. Poole *et al.*, J. Phys. Condens. Matter **17**, L431 (2005).
[18] M. A. Anisimov *et al.*, in *Aqueous System at Elevated Temperatures and Pressures: Physical Chemistry in Water, Steam and Hydrothermal Solutions*, edited by D. A. Palmer *et al.* (Elsevier, Amsterdam, 2004), pp. 29–71.
[19] K. Morishige and K. Kawano, J. Chem. Phys. **110**, 4867 (1999).
[20] L. Liu *et al.*, Phys. Rev. Lett. **95**, 117802 (2005).
[21] S.-H. Chen *et al.*, Proc. Natl. Acad. Sci. U.S.A. **103**, 9012 (2006).
[22] E. Lindahl *et al.*, J. Mol. Model. **7**, 306 (2001).
[23] P. J. Artymiuk *et al.*, Acta Crystallogr. B **38**, 778 (1982).
[24] H. R. Drew *et al.*, Proc. Natl. Acad. Sci. U.S.A. **78**, 2179 (1981).
[25] J. Wang *et al.*, J. Comput. Chem. **21**, 1049 (2000); E. J. Sorin and V. S. Pande, Biophys. J. **88**, 2472 (2005).
[26] M. W. Mahoney and W. L. Jorgensen, J. Chem. Phys. **112**, 8910 (2000).
[27] The TIP5P model was shown to accurately reproduce the thermodynamic properties of water at ambient conditions [26]. The properties of TIP5P in the supercooled region were studied in M. Yamada *et al.*, Phys. Rev. Lett. **88**, 195701 (2002); and D. Paschek, Phys. Rev. Lett. **94**, 217802 (2005), where it was shown that TIP5P reproduces the dynamic and thermodynamic anomalies of water.
[28] To fit the data, we use a prediction of the ''mode coupling theory'' (MCT) [32] at high T with parameters $T_{\mathrm{MCT}} \approx 227$ and $\gamma \approx 2.72$
[29] J. Horbach and W. Kob, Phys. Rev. B **60**, 3169 (1999).
[30] S. S. Ashwin and S. Sastry, J. Phys. Condens. Matter **15**, S1253 (2003).
[31] F. W. Starr *et al.*, Physica (Amsterdam) **323A**, 51 (2003).
[32] W. Götze, J. Phys. Condens. Matter **11**, A1 (1999).
[33] The error bars in the enthalpy calculation do not exceed 1%. To assess the error bar on the position of the maximum, we perform 1000 fittings of the data points by adding random Gaussian variables. The standard deviation of the Gaussian noise was taken to be the error bars in enthalpy.
[34] The $\langle x^2 \rangle$ for DNA was calculated in the same fashion as for lysozyme.
[35] The values of C_P are different in each case with $C_P^{\mathrm{DNA}}(T_W) > C_P^{\mathrm{protein}}(T_W)$, probably because the DNA hydration level (368%) is larger than the lysozyme hydration level (156%) and the fact that the specific heat of water is larger than protein and DNA.
[36] The difference in the height of the first peak of $g(r)$ for lysozyme and DNA hydration water is due to the different hydration levels of the two systems.
[37] J. R. Errington and P. G. Debenedetti, Nature (London) **409**, 318 (2001).
[38] S.-H. Chen *et al.*, J. Chem. Phys. (to be published).
[39] T. Morishita, Phys. Rev. Lett. (to be published).
[40] A. L. Lee and A. J. Wand, Nature (London) **411**, 501 (2001).
[41] D. Vitkup *et al.*, Nat. Struct. Biol. **7**, 34 (2000).
[42] W. Doster and M. Settles, in *Hydration Processes in Biology*, edited by M. C. Bellissent-Funel (IOS Press, Amsterdam, 1999), pp. 177–191.
[43] G. Caliskan *et al.*, J. Am. Chem. Soc. **128**, 32 (2006).
[44] J. E. Curtis *et al.*, J. Am. Chem. Soc. **126**, 15 928 (2004).
[45] V. Réat *et al.*, Proc. Natl. Acad. Sci. U.S.A. **97**, 9961 (2000).
[46] M. Teeter *et al.*, Proc. Natl. Acad. Sci. U.S.A. **98**, 11 242 (2001).
[47] M. Weik *et al.*, Protein Science **10**, 1953 (2001).
[48] P. W. Fenimore *et al.*, Proc. Natl. Acad. Sci. U.S.A. **101**, 14 408 (2004).
[49] P. W. Fenimore *et al.*, Physica (Amsterdam) **351A**, 1 (2005).