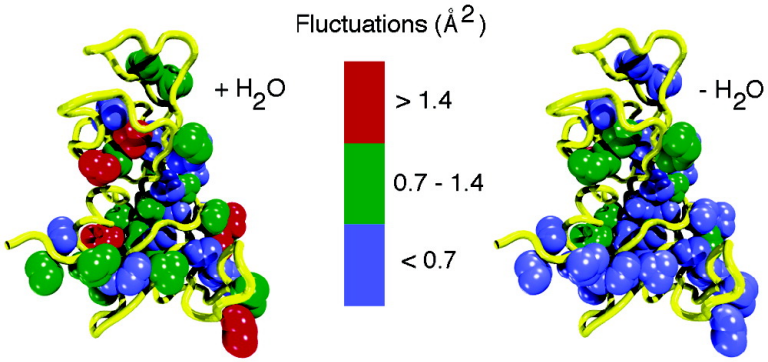
Communication

Methyl Group Dynamics as a Probe of the Protein Dynamical Transition

Joseph E. Curtis, Mounir Tarek, and Douglas J. Tobias

J. Am. Chem. Soc., **2004**, 126 (49), 15928-15929• DOI: 10.1021/ja0480623 • Publication Date (Web): 13 November 2004



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 4 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S
COMMUNICATIONS

Published on Web 11/13/2004

Methyl Group Dynamics as a Probe of the Protein Dynamical Transition

Joseph E. Curtis,[§] Mounir Tarek,[‡] and Douglas J. Tobias*,[†]

NIST Center for Neutron Research, National Institute of Standards and Technology, Gaithersburg, Maryland 20899, Equipe de dynamique des assemblages membranaires, UMR CNRS/UHP 7565, Université Henri Poincaré, 54506 Vandoeuvre-lès-Nancy, France, and Department of Chemistry and Institute for Surface and Interface Science, University of California, Irvine, California 92697-2025

Received April 4, 2004; E-mail: dtobias@uci.edu

As the temperature is raised above ~200 K, hydrated proteins undergo a change in dynamics, the so-called dynamical transition,[1] from glasslike, in which the motion is primarily vibrational, to liquidlike, in which transitions between taxonomic substates are possible. The increase in motion accompanying the transition has been observed by techniques that probe different parts of a protein molecule over a wide range of time scales, including Mössbauer spectroscopy,[2,3] X-ray crystallography,[4] neutron scattering,[5,6] light scattering,[7] nuclear magnetic resonance (NMR) spectroscopy,[8] and molecular dynamics (MD) simulations.[9,10] Numerous studies have demonstrated that the transition is dependent on solvent: in the absence of solvent it is suppressed, and as the solvent viscosity is increased, the transition temperature increases and the magnitude of nonharmonic motions is attenuated.[3,6,7] However, it has recently been argued that the transition can be predicted by extrapolating the high T motion (probed by NMR) of a subset of side chains to low T, i.e., it does not involve a purely global transition in the protein−solvent system.[8] The extent to which solvent affects protein dynamics has implications for fundamental studies aimed at elucidating the role of specific protein motions in protein function, and practical applications to biopreservation by suppression of protein function and degradation pathways.[11] We report the temperature dependence of methyl group dynamics predicted by MD simulations of a protein over a range of temperatures spanning the dynamical transition, with and without aqueous solvent. Our results confirm that solvent is required for the dynamical transition and demonstrate that the transition is accompanied by an abrupt increase in mobility throughout most of the protein.

The results were derived from 2-ns MD trajectories of the protein ribonuclease A (RNase) in a hydrated crystal (2 RNase molecules plus 817 waters) and a dehydrated powder (8 RNase molecules),[12] at 25 K increments from 100 to 300 K, and a single RNase molecule in solution (3453 waters) at 300 K. The structures of the protein molecules in the dehydrated powder differ only slightly from those in the crystal (1−1.5 Å C^{α} rmsd).[12] The simulation protocols, described in detail elsewhere,[10,12] have produced both protein and water dynamics in excellent agreement with neutron scattering data. Methyl group dynamics were characterized in terms of mean-squared fluctuations (MSFs) of H atoms, which are dominated by libration/rotation of C−H bond vectors. The MSFs are therefore useful for discussing the motion probed by deuterium NMR relaxation data on labeled methyl groups and incoherent neutron scattering data that are dominated by contributions from nonexchangeable H atoms. The MSFs were accumulated in blocks of 100 ps, which is intermediate between the time scales probed by NMR relaxation in small proteins (~1 ns) and elastic neutron scattering (<100 ps).

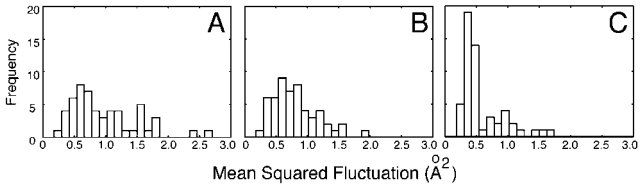


Figure 1. Histograms of methyl proton mean-squared fluctuations in RNase from MD simulations at 300 K: (A) solution, (B) crystal, (C) dehydrated powder.

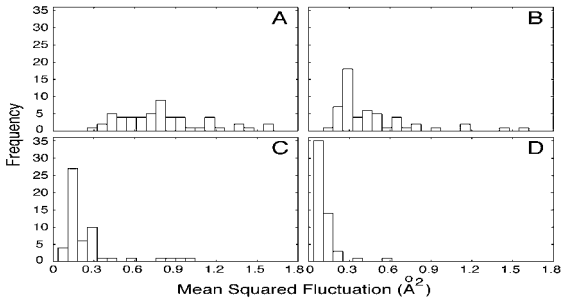


Figure 2. Histograms of methyl proton mean-squared fluctuations in hydrated RNase crystal: (A) 300 K, (B) 250 K, (C) 200 K, (D) 150 K.

Figure 1 displays histograms of methyl MSFs for RNase at 300 K in solution, in the crystal where the protein is well hydrated, and in a dehydrated powder. In solution the MSFs span a relatively wide range, indicating a marked degree of dynamical heterogeneity, consistent with NMR.[8] The MSF distribution in the hydrated crystal (Figure 1B) is qualitatively similar to the solution result, except for a slight shift to lower values. Dehydration results in a dramatic shift of the MSFs to lower values (Figure 1C). Thus, it is clear that water is required for the methyl groups to exhibit the full range of motion characteristic of the liquidlike protein state at room temperature.

In Figure 2, histograms of the methyl MSFs in the hydrated crystal are plotted at several temperatures bracketing the dynamical transition (~200 K). The methyl group fluctuations shift systematically toward lower values as the temperature is lowered, i.e., the dynamics of most of the methyl groups exhibit a qualitatively similar temperature dependence.

The temperature dependence of the MSFs of individual methyl groups in the hydrated RNase crystal and the dehydrated RNase powder is plotted in Figure 3. In the hydrated system, the MSFs generally increase linearly with T below the dynamical transition temperature of ~200 K, and the majority show an abrupt increase with temperature above 200 K. In contrast, in the dehydrated system, the MSFs generally follow a roughly linear increase over the entire temperature range (i.e. do not undergo a dynamical transition). Figure 3A shows that the dynamical transition, reported by the methyl groups, occurs throughout the hydrated protein, at

[§] National Institute of Standards and Technology.
[‡] Université Henri Poincaré.
[†] University of California, Irvine.

10.1021/ja0480623 CCC: $27.50

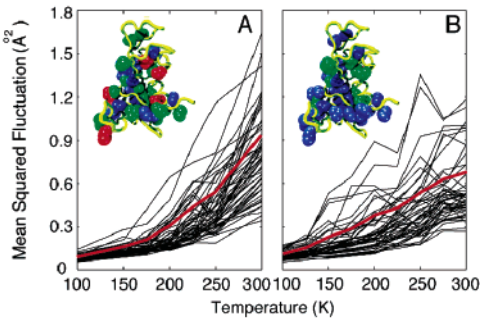


Figure 3. MSFs of methyl protons from MD simulations of RNase, plotted separately for each methyl group (excluding methionine): (A) hydrated (crystal), (B) dehydrated (powder). The thick red lines are averages. Insets show the crystal structure with methyl groups colored by MSF values at 300 K (>1.4 Å^2, red; 0.7−1.4 Å^2, green; <0.7 Å^2, blue).

roughly the same T (∼200 K for most methyl groups), albeit with a degree depending on sequence, but not on solvent accessibility, while the transition is suppressed throughout most of the protein in the absence of solvent.

We discuss our results in light of a recently reported new view of the dynamical transition following a brief summary of the data on which it is based.[8] C−D order parameters (S^2),[13] indicative of mobility on the nanosecond time scale, were determined by deuterium quadrupolar relaxation for each of the resolvable methyl groups in a calmodulin−peptide complex over a limited range of T (288−346 K) well above the transition in solution.[8] The S^2 values fell roughly into three classes corresponding to low, intermediate, and high mobility. By assuming a linear (harmonic model) dependence of S^2 on T, and extrapolating to low T, a plot of an average "disorder" parameter ($1-S^2$) exhibited an abrupt increase at ∼200 K reminiscent of the dynamical transition. On the basis of the T dependence of the motion in the three classes, the transition was attributed to the activation of methyls with intermediate mobility, without invoking a global transition in the protein−solvent system.[8]

When a wide range of T (50−300 K) spanning the transition is considered, the whole distribution of methyl group fluctuations in the hydrated protein (crystal) shifts to lower mobility (Figure 2). This is in accord with the NMR data obtained over a limited high T range. However, we find that most of the methyl groups in the hydrated protein display a nonlinear T dependence with a dynamical transition at ∼200 K (Figure 3A), in contrast to the linear dependence assumed in the order parameter model. In addition, most methyl groups do not undergo a transition in a dehydrated protein (Figure 3B), which was not considered in the NMR study.

Our demonstration that water is required for the structural relaxation of a protein above its dynamical transition temperature agrees with many previous studies.[3,6,7] The crucial role of solvent in protein dynamics is further supported by the observation that the transition temperature is increased, and the additional motion (beyond harmonic) accompanying the transition is attenuated, by immersing proteins in more viscous media, such as glycerol, sucrose, and trehalose.[3,6,7] Our study provides direct evidence that both the amplitudes and distribution of motion are influenced by the solvent and is not exclusively an intrinsic property of the protein. Thus, it is often said that protein motion is slave to solvent motion.[14] An alternative point of view is that solvent acts as a plasticizer that allows the activation of protein motions by decreasing the local viscosity of protein atoms.[15] The mechanism of plasticization involves relaxation of the protein−solvent hydrogen bond network[15] via solvent translational motion.[16]

Acknowledgment. This work was supported by the National Science Foundation (Grant MCB-0078278).

Supporting Information Available: Data plotted in Figure 3. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Doster, W.; Cusack, S.; Petry, W. *Nature (London)* **1989**, *337*, 754−756.
(2) (a) Knapp, E. W.; Fischer, S. F.; Parak, F. *J. Phys. Chem.* **1982**, *86*, 5042−5047. (b) Keller, H.; Debrunner, P. G. *Phys. Rev. Lett.* **1980**, *45*, 68−71. (c) Parak, F.; Frolov, E. N.; Mössbauer, R. L.; Goldanskii, V. I. *J. Mol. Biol.* **1981**, *145*, 825−833. (d) Parak, F.; Knapp, E. W.; Kucheida, D. *J. Mol. Biol.* **1982**, *161*, 177−194. (e) Melchers, B.; Knapp, E. W.; Parak, F.; Cordone, L.; Cupane, A.; Leone, M. *Biophys. J.* **1996**, *70*, 2092−2099.
(3) Lichtenegger, H.; Doster, W.; Kleinert, T.; Birk, A.; Sepiol, B.; Vogl, G. *Biophys. J.* **1999**, *76*, 414−422.
(4) (a) Frauenfelder, H.; Petsko, G. A.; Tsernoglou, D. *Nature (London)* **1979**, *280*, 558−563. (b) Tilton, R. F. Jr.; Dewan, J. C.; Petsko, G. A. *Biochemistry* **1992**, *31*, 2469−2481. (c) Teeter, M. M.; Yamano, A.; Stec, B.; Mohanty, U. *Proc. Natl. Acad. Sci. U.S.A.* **2001**, *98*, 11242−11247.
(5) (a) Rorshach, H. E.; Bearden, D. W. *Scanning Microsc.* **1987**, *1*, 2043−2049. (b) Andreani, C.; Filabozzi, A.; Menzinger, F.; Desideri, A.; Deriu, A.; DiCola, D. *Biophys. J.* **1995**, *68*, 2519−2523. (c) Fitter, J.; Lechner, R. E.; Büldt, G.; Dencher, N. A. *Phys. B* **1996**, *226*, 61−65. (d) Fitter, J.; Lechner, R. E.; Dencher, N. A. *Biophys. J.* **1997**, *73*, 2126−2137. (e) Réat, V.; Patzelt, H.; Ferrand, M.; Pfister, C.; Oesterhelt, D.; Zaccaï, G. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4970−4975. (f) Daniel, R. M.; Smith, J. C.; Ferrand, M.; Héry, S.; Dunn, R.; Finney, J. L. *Biophys. J.* **1998**, *75*, 2504−2507.
(6) (a) Ferrand, M.; Dianoux, A. J.; Petry, W.; Zaccaï, G. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9668−9672. (b) Fitter, J. *Biophys. J.* **1999**, *76*, 1034−1042. (c) Cordone, L.; Ferrand, M.; Vitrano, E.; Zaccaï, G. *Biophys. J.* **1999**, *76*, 1043−1047. (d) Tsai, A. M.; Neumann, D. A.; Bell, L. N. *Biophys. J.* **2000**, *79*, 2728−2732. (e) Paciaroni, A.; Cinelli, S.; Onori, G. *Biophys. J.* **2002**, *83*, 1157−1164.
(7) Caliskan, G.; Kisliuk, A.; Sokolov, A. P. *J. Non-Cryst. Solids* **2002**, *307−310*, 868−873.
(8) Lee, A. L.; Wand, J. *Nature (London)* **2001**, *411*, 501−504.
(9) (a) Wong, C. F.; Zheng, C.; McCammon, J. A. *Chem. Phys. Lett.* **1989**, *154*, 151−154. (b) Smith, J. C.; Kuczera, K.; Karplus, M. *Proc. Natl. Acad. Sci. U.S.A.*, **1990**, *90*, 9135−9139. (c) Loncharich, R. J.; Brooks, B. R. *J. Mol. Biol.* **1990**, *215*, 439−455. (d) Arcangeli, C.; Bizzarri, A. R.; Cannistraro, S. *Chem. Phys. Lett.* **1998**, *291*, 7−14. (e) Simon, C.; Aalouach, M.; Smith, J. C. *Faraday Discuss.* **1998**, *111*, 95−102. (f) Steinbach, P. J.; Loncharich, R. J.; Brooks, B. R. *Chem. Phys.* **1991**, *158*, 383−394. (g) Melchionna, S.; Falconi, M.; Desideri, A. *J. Chem. Phys.* **1998**, *108*, 6033−6041. (h) Paciaroni, A.; Stroppolo, M. E.; Arcangeli, C.; Bizzarri, A. R.; Desideri, A.; Cannistraro, S. *Eur. Biophys. J.* **1999**, *28*, 447−456. (i) Vitkup, D.; Ringe, D.; Petsko, G. A.; Karplus, M. *Nat. Struct. Biol.* **2000**, *7*, 34−38. (j) Tournier, A. L.; Xu, J.; Smith, J. C. *PhysChemComm* **2003**, *6*, 6−8.
(10) Tarek, M.; Tobias, D. J. *J. Am. Chem. Soc.* **2000**, *122*, 10450−10451.
(11) (a) Storey, K. B.; Storey, J. M. *Sci. Am.* **1990**, *263*, 92−97. (b) Fox, K. C. *Science* **1995**, *267*, 1922−1923.
(12) (a) Tarek, M.; Tobias, D. J. *J. Am. Chem. Soc.* **1999**, *121*, 9740−9741. (b) Tarek, M.; Tobias, D. J. *Biophys. J.* **2000**, *79*, 3244−3257.
(13) Lipari, G.; Szabo, A. *J. Am. Chem. Soc.* **1982**, *104*, 4546−4559.
(14) (a) Frauenfelder, H.; Fenimore, P. W.; McMahon, B. H. *Biophys. Chem.* **2002**, *98*, 35−48. (b) Fenimore, P. W.; Frauenfelder, H.; McMahon, B. H.; Parak, F. G. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 16047−16051.
(15) Doster, W.; Settles, M. In *Hydration Processes in Biology*; Bellissent-Funel, M. C., Ed.; IOS Press: Amsterdam, 1999; Vol. 305, pp 177−191.
(16) Tarek, M.; Tobias, D. J. *Phys. Rev. Lett.* **2002**, *88*, 138101.

JA0480623