J|A|C|S COMMUNICATIONS

Published on Web 03/19/2010

# The Low-Temperature Inflection Observed in Neutron Scattering Measurements of Proteins Is Due to Methyl Rotation: Direct Evidence Using Isotope Labeling and Molecular Dynamics Simulations

Kathleen Wood,*,†,⊥ Douglas J. Tobias,*,‡ Brigitte Kessler,§ Frank Gabel,¶ Dieter Oesterhelt,§ Frans A. A. Mulder,|| Giuseppe Zaccai,† and Martin Weik¶

*Institut Laue Langevin, Grenoble Cedex 9, France, Australian Nuclear Science and Technology Organisation, Menai NSW Australia, Department of Chemistry, University of California, Irvine, California, Max Planck Institute of Biochemistry, Martinsried, Germany, CEA, IBS, Laboratoire de Biophysique Moleculaire, F-38054 Grenoble, France, CNRS, UMR5075, F-38027, Grenoble, France, Universite Joseph Fourier, F-38000, Grenoble, France, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, The Netherlands*

Received December 13, 2009; E-mail: kwo@ansto.gov.au; dtobias@uci.edu

Biological macromolecules are animated by motions occurring over a wide range of time and length scales, and an understanding of the correlation between their dynamics and functions remains a challenge. A first step is to characterize protein dynamical complexity and identify the types of motions contributing to measurements. Several papers have underlined the important contribution of methyl groups[1–7] to measured protein dynamics.

Protein dynamics is a signature of its underlying energy landscape, typically explored through analysis of temperature-dependent data.[8] The so-called protein dynamical transition between 180 and 250 K, observed as a deviation from linear behavior in atomic mean square displacements (MSD) as a function of temperature, has been discussed extensively in this context.[9,10] It is present only in samples hydrated to a sufficient degree.[9,11,12] A second break from linear behavior, present in both dry and hydrated samples, was then observed in high-resolution neutron scattering data[13–15] at 120−150 K, which was also reproduced by molecular dynamics simulations.[16] The low-temperature inflection was found to be absent in both tRNA[17] and hydration water[18–20] and was attributed to the onset of methyl-group rotations through analysis of molecular dynamics (MD) simulations.[2,21] Elastic incoherent neutron scattering (EINS) studies on homomeric polypeptide model systems provided experimental evidence that the low-temperature inflection is related to methyl rotation.[22] Others have linked it to the onset of interfacial water rotational motions.[23]

Here, we investigated the contribution of methyl groups to purple membrane (PM) dynamics as measured by EINS, by using isotope labeling and MD simulations, and comparison to NMR results. PM is formed predominantly of a single membrane protein, bacteriorhodopsin (BR), and various lipid species. EINS is sensitive to hydrogen atoms and generally provides information averaged over the whole system. Using hydrogen−deuterium isotope labeling it is possible to focus on one part of a complex sample. In previous work, where deuterated PM samples were produced with either hydrogenated leucine or isoleucine residues, both methyl-containing side chains, an inflection was observed at 120−130 K[24] (see Figure S1, Supporting Information). In the present study we therefore measured the dynamics of a non-methyl-containing side chain, lysine, in BR by studying a completely deuterated PM in which the lysine residues are hydrogenated and compared these dynamics to the dynamics of a natural abundance control PM sample. We take the lysine side chain as representative of non-methyl-containing moieties in the protein.

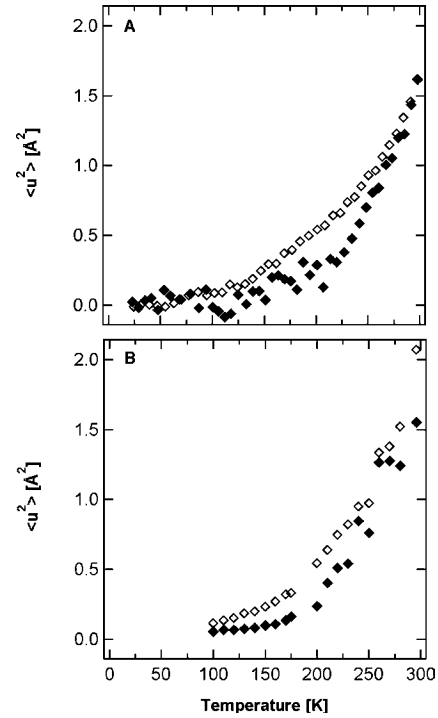


***Figure 1.*** MSD from neutron scattering measurements (A) and molecular dynamics simulations (B). In A, natural abundance PM is shown as open diamonds, and deuterated PM with hydrogenated lysine residues is shown as filled diamonds. In B, analysis of the simulations is performed at each temperature for all nonexchangeable PM hydrogen atoms (open diamonds) and for lysine residues only (full diamonds).

Natural abundance PM (H-PM) and deuterated PM with hydrogenated lysine residues (Lys-PM) were purified and hydrated to 86% relative humidity. Neutron scattering experiments were performed on the IN16 backscattering spectrometer at the Institut Laue Langevin, Grenoble, with an energy resolution of 0.9 $\mu$eV and an accessible wavevector ($Q$) range of 0.02−1.9 Å$^{-1}$. The elastically scattered neutrons were recorded on heating from 20 to 300 K and MSD were extracted.

Figure 1A represents atomic MSD on the nanosecond time scale extracted from neutron scattering measurements for the H-PM and

† Institut Laue Langevin.
⊥ Australian Nuclear Science and Technology Organisation.
‡ University of California.
§ Max Planck Institute of Biochemistry.
¶ CEA, IBS, Laboratoire de Biophysique Moleculaire; CNRS, UMR5075; and Universite Joseph Fourier.
|| University of Groningen.


10.1021/ja910502g 

Lys-PM. In the first, the contributions to the incoherent scattering cross section are as follows: 73% BR, 26% lipids and 1% hydration $D_2O$, with hydrogen atoms in methyl groups contributing 34%. In the Lys-PM, the scattering from the membrane is in part masked: 53% comes from the labeled lysine residues, 46% from the deuterated membrane (15% of the scattering cross section is from deuterated methyl groups), and 1% hydration $D_2O$. The MSD of H-PM are similar to those of the specifically labeled sample at low temperature. A deviation from linear behavior is observed in H-PM data at approximately 125 K, also observed in the same temperature range in labeled leucine and isoleucine samples[24] (Figure S1, Supporting Information). Such a break is strongly attenuated in the Lys-PM: MSD increase smoothly up to ~200 K, where they begin to have larger amplitudes. At approximately 250 K Lys-PM and H-PM MSD again become similar. The inflection observed at ~125 K in H-PM is absent when mainly the dynamics of a non-methyl-containing side chain is probed, and the data provide experimental evidence that the inflection observed in protein MSD measured on high-resolution spectrometers is due to methyl group dynamics.

In order to assign the contributions to the elastic neutron scattering to individual groups of atoms, molecular dynamics simulations were performed on a PM model at 13 temperatures spanning 100 to 296 K. The PM model was taken from[25] and is constituted of a single BR trimer with 28 lipid and 1924 water molecules. The simulations were equilibrated at each temperature for several nanoseconds and production runs of 5 ns performed.

Atomic MSD calculated over the entire trajectories are shown in Figure 1B. The results quantitatively reproduce the experimental data, although the low-temperature inflection is at about ~50 K higher than in the neutron MSD. This discrepancy is consistent with results from other experimental and MD studies of methyl group dynamics, where evidence was provided that the barriers to methyl rotations in the force field are too high[26] and that sampling in the MD may be incomplete at low temperature.[4] Since the same trends in temperature dependence are observed here in the simulation and scattering results, the simulations were further analyzed to explain the origin of the apparent transition. Plotting MSD for hydrogen atoms in methyl groups and MSD for all hydrogen atoms excluding methyl groups indeed confirms methyl group dynamics are at the origin of the inflection (Figure S2, Supporting Information). MSD from methyl group hydrogen atoms are then analyzed using several different averaging times (100 ps and 1 and 5 ns) to mimic different instrument resolutions and are plotted in Figure S3 (Supporting Information). As seen in Figure S3, the longer the time frame, the lower the temperature at which the apparent onset of methyl dynamics occurs. The analysis predicts that the inflection due to methyl groups observed in MSD measured by neutron scattering would be shifted to a higher temperature when using instruments with lower-energy resolutions. Such a shift is observed when H-PM samples are measured on a spectrometer of 10 $\mu$eV resolution.[27]

Further experimental evidence for the methyl-associated inflection can be gleaned from NMR: leucine, valine, alanine, and threonine methyl groups, together responsible for 80% of all methyl groups in proteins, undergo 3-fold jump rotations with time constants of 30−80 ps at room temperature.[4,28] Temperature-dependent solid state NMR measurements on BR[28] (shown in Figure S4, Supporting Information) indicate that at ~150 K the valine, leucine, and threonine methyl groups undergo jumps on the nanosecond time scale. The NMR data plotted in Figure S4[28] predict that, at the 0.9 $\mu$eV resolution of the IN16 neutron spectrometer, methyl rotations will no longer contribute to the scattering below 100 K. The NMR data further predict that the inflection takes place at a lower temperature for isoleucine compared to that for leucine, a fact that has been experimentally verified by our EINS data of labeled leucine and isoleucine PM (Figure S1, Supporting Information).[24] In conclusion, NMR suggests it is the rotational part of methyl dynamics that is at the origin of the MSD inflection at 125 K in neutron data.

Assuming an Arrhenius temperature dependence of methyl group rotations down to 100 K, NMR methyl correlation times in the literature and the time-dependent analysis of MD simulations presented here both provide evidence that the observed low-temperature inflection is an effect of finite instrumental energy resolution[29] and not due to an 'onset' of dynamics.[2,21] Models involving the onset of dynamics are apt when alternate states become depleted or populated. On the other hand the relative energies of the three states of the methyl group are isoenergetic, and the apparent dynamics will always be present, unless the kinetics are too slow for the observing technique.

**Acknowledgment.** The study was supported by the EU DLAB program under Contracts HPRI-CT-2001-50035 and RII3-CT-2003-505925. D.J.T. thanks the UJF for a visiting professorship, and the NSF for funding (Grant CHE-0750175). M.W. and F.A.A.M. thank EGIDE and the NWO for funding.

**Supporting Information Available:** Details of sample preparation, neutron scattering experiments, and molecular dynamics simulations. This material is available free of charge via the Internet at http://pubs.acs.org.

## References

(1) Lee, A. L.; Wand, A. J. *Nature* **2001**, *411*, 501.
(2) Roh, J. H.; Novikov, V. N.; Gregory, R. B.; Curtis, J. E.; Chowdhuri, Z.; Sokolov, A. P. *Phys. Rev. Lett.* **2005**, *95*, 038101.
(3) Curtis, J. E.; Tarek, M.; Tobias, D. J. *J. Am. Chem. Soc.* **2004**, *126*, 15928.
(4) Best, R. B.; Clarke, J.; Karplus, M. *J. Mol. Biol.* **2005**, *349*, 185.
(5) Best, R. B.; Clarke, J.; Karplus, M. *J. Am. Chem. Soc.* **2004**, *126*, 7734.
(6) Doster, W. *Eur. Biophys. J. Biophys. Lett.* **2008**, *37*, 591.
(7) Krishnan, M.; Kurkal-Siebert, V.; Smith, J. C. *J. Phys. Chem. B* **2008**, *112*, 5522.
(8) Frauenfelder, H.; Sligar, S. G.; Wolynes, P. G. *Science* **1991**, *254*, 1598.
(9) Doster, W.; Cusack, S.; Petry, W. *Nature* **1989**, *337*, 754.
(10) Tournier, A. L.; Smith, J. C. *Phys. Rev. Lett.* **2003**, *91*, 208106.
(11) Ferrand, M.; Dianoux, A. J.; Petry, W.; Zaccai, G. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 9668.
(12) Fitter, J. *Biophys. J.* **1999**, *76*, 1034.
(13) Reat, V.; Patzelt, H.; Ferrand, M.; Pfister, C.; Oesterhelt, D.; Zaccai, G. *Proc. Natl. Acad. Sci. U.S.A.* **1998**, *95*, 4970.
(14) Gabel, F.; Weik, M.; Doctor, B. P.; Saxena, A.; Fournier, D.; Brochier, L.; Renault, F.; Masson, P.; Silman, I.; Zaccai, G. *Biophys. J.* **2004**, *86*, 3152.
(15) Gabel, F.; Weik, M.; Masson, P.; Renault, F.; Fournier, D.; Brochier, L.; Doctor, B. P.; Saxena, A.; Silman, I.; Zaccai, G. *Biophys. J.* **2005**, *89*, 3303.
(16) Hayward, J. A.; Smith, J. C. *Biophys. J.* **2002**, *82*, 1216.
(17) Roh, J. H.; Briber, R. M.; Damjanovic, A.; Thirumalai, D.; Woodson, S. A.; Sokolov, A. P. *Biophys. J.* **2009**, *98*, 2755.
(18) Wood, K.; Plazanet, M.; Gabel, F.; Kessler, B.; Oesterhelt, D.; Tobias, D. J.; Zaccai, G.; Weik, M. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 18049.
(19) Wood, K.; Frolich, A.; Paciaroni, A.; Moulin, M.; Hartlein, M.; Zaccai, G.; Tobias, D. J.; Weik, M. *J. Am. Chem. Soc.* **2008**, *130*, 4586.
(20) Gabel, F.; Bellissent-Funel, M. C. *Biophys. J.* **2007**, *92*, 4054.
(21) Roh, J. H.; Curtis, J. E.; Azzam, S.; Novikov, V. N.; Peral, I.; Chowdhuri, Z.; Gregory, R. B.; Sokolov, A. P. *Biophys. J.* **2006**, *91*, 2573.
(22) Schiro, G.; Caronna, C.; Natali, F.; Cupane, A. *J. Am. Chem. Soc.* **2010**, *132*, 1371.
(23) Zanotti, J. M.; Gibrat, G.; Bellissent-Funel, M. C. *Phys. Chem. Chem. Phys.* **2008**, *10*, 4865.
(24) Wood, K.; Grudinin, S.; Kessler, B.; Weik, M.; Johnson, M.; Kneller, G. R.; Oesterheit, D.; Zaccai, G. *J. Mol. Biol.* **2008**, *380*, 581.
(25) Baudry, J.; Tajkhorshid, E.; Molnar, F.; Phillips, J.; Schulten, K. *J. Phys. Chem. B* **2001**, *105*, 905.
(26) Chatfield, D. C.; Wong, S. E. *J. Phys. Chem. B* **2000**, *104*, 11342.
(27) Lehnert, U.; Reat, V.; Weik, M.; Zaccai, G.; Pfister, C. *Biophys. J.* **1998**, *75*, 1945–52.
(28) Keniry, M. A.; Kintanar, A.; Smith, R. L.; Gutowsky, H. S.; Oldfield, E. *Biochemistry* **1984**, *23*, 288.
(29) Khodadadi, S.; Pawlus, S.; Roh, J. H.; Sakai, V. G.; Mamontov, E.; Sokolov, A. P. *J. Chem. Phys.* **2008**, *128*, 195106.

JA910502G