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Effects of hydration water on protein methyl group dynamics in solution

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Elastic and quasielastic neutron scattering experiments have been used to investigate the dynamics of methyl groups in a protein-model hydrophobic peptide in solution. The results suggest that, when the hydrophobic side chains are hydrated by a single hydration water layer, the only allowed motions are confined and attributed to librational and rotational movement associated with the methyl groups. They provide unique experimental evidence that the structural and dynamical properties of the interfacial water strongly influence the side-chain dynamics and the activation of diffusive motion.

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In this Rapid Communication we consider protein-model molecules in aqueous solution and ask how the dynamics of the surface solvent affects the side chain motions that trigger structural relaxation of the protein when the temperature is raised above the dynamical transition temperature (~200 K) [1]. Above that temperature and a critical hydration level, water seems to act as a lubricant to protein motions, enabling exploration of a larger conformational space. Using molecular dynamics simulations, Curtis *et al.* [2] have studied the temperature (T) and hydration (h) dependences of methyl-group mean-square fluctuations (MSFs) in a hydrated protein crystal. They confirm that a critical amount of solvent is required for there to be a dynamical transition, and they demonstrate that the dynamical transition is accompanied by an increase in mobility throughout most of the protein. While only rotational motions of side chains are active when the system is glassy, translational displacements become active and dominate when the protein-water complex becomes liquidlike [3]. Many other studies using both experiments and simulations have contributed important information about hydrated protein and hydration water dynamics [4]. Here we report elastic scattering and quasielastic neutron scattering (QENS) experiments to probe, in solution, the crucial influence of the first hydration layer on the intrinsic molecule dynamics (internal dynamics) in a simplified protein-model interface The structure and dynamics of the first layer water network are influenced by the protein interface [4]. The first layer's dynamics is not relaxed as are those of the outer layers (approaching bulk water dynamics), but instead is comparable to the dynamics of supercooled water. The properties of the surface water are intimately connected to protein stability and function, hence the importance of studying a simple biological system in solution, with a single hydration shell. We consider the picosecond dynamics, as a function of T, of the solute N-acetyl-leucine-methylamide (NALMA), which comprises a hydrophobic amino-acid side chain $(CH_3)_2$-CH-CH_2 attached to the $C_{\acute{\alpha}}$ atom of a polar-blocked polypeptide backbone with CH_3 end caps (CH_3-CO-NH-$C_{\acute{\alpha}}$H-CO-NH-CH_3). Results for a similar but completely hydrophilic model interface, N-acetyl-glycine-methylamide (NAGMA), are presented for comparison. NAGMA peptide does not have an extended side chain (CH), and its internal dynamics is dominated by the CH_3 end caps.

In order to study the solute dynamics we performed experiments on hydrogenated NALMA (NAGMA) in D_2O solution at 2M (3M) concentration, having exchanged NH into ND. At these high concentrations (~2.4 g H_2O per gram of solute) the water molecules form a single hydration layer shared among solute molecules [5]. Previous experimental and theoretical studies have demonstrated that in solution these molecules organize as isolated entities or as very small clusters [5]. Our results prove that a hydrophobic side chain requires more than a single layer of solvent in order to attain the liquidlike dynamical regime. We show that when hydrophobic side chains are hydrated by a single water layer, the only allowed motions are confined and attributed to simple rotations of methyl groups.

The QENS experiment was performed using the Disk Chopper time-of-flight Spectrometer (DCS) [6] at the NIST Center for Neutron Research (NCNR). In order to cover a large dynamical range (1–50 ps), experiments were carried out using full width at half maximum (FWHM) energy resolutions of 35 μeV and 90 μeV. Measurements were performed at 281 K and at room temperature T_R. The elastic experiment was performed using the backscattering spectrometer IN13 [7] at the Institut Laue Langevin (ILL), with an energy resolution of 8 μeV (time scale ~80 ps), between 275 and 310 K. The elastic and quasielastic spectra were corrected for sample holder scattering and normalized using vanadium standards. All the spectra were corrected for scattering from the buffer, subtracting the D_2O scattering with a factor which correctly accounts for the proportions of solute and solvent in the solution. The reduced QENS data were analyzed using the NCNR program DAVE [8] as reported elsewhere [5].

The higher-resolution QENS data largely provide information about the translational dynamics. At 281 K the dependence on wave vector transfer Q of the half width at half maximum (HWHM) translational linewidth, $\Gamma(Q)$, follows hydrodynamic regime behavior, yielding a diffusion coefficient of 1.3×10^{-6} cm^2/s. A second dynamical component,

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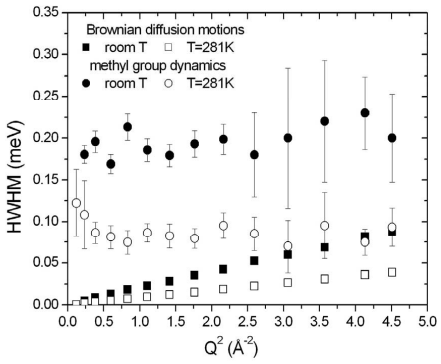

FIG. 1. Half widths at half maximum (HWHMs) plotted versus Q^2 for 2 M NALMA in heavy water at 281 K (open symbols) and T_R (solid symbols) inferred from low-resolution data. The narrower half widths (squares) are associated with Brownian diffusion of the molecules in solution. The broader half widths (circles) are associated with localized methyl-group motions.

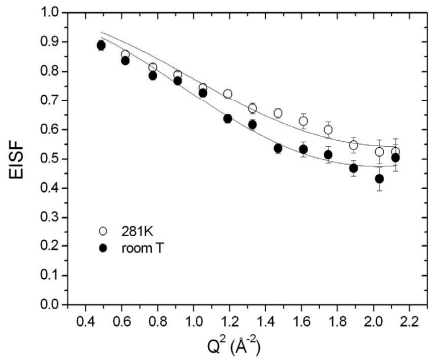

FIG. 2. Elastic incoherent structure factors (EISFs) for 2 M NALMA in D_2O solution at 281 K (open symbols) and T_R (solid symbols). The solid lines are fits using the three-site jump rotational model.

attributed to the internal dynamics, was also observed but was better determined from the lower-resolution data as described in [5]. The lower-resolution data provide information about the faster internal dynamics. The inferred HWHM, at 281 K, is independent of Q with an average value of 0.08 meV. The analysis of the lower-resolution spectra, at T_R, was also performed using known values of the NALMA Brownian diffusion coefficient, 3×10^{-6} cm^2/s [5]. In agreement with the 281 K observations, the HWHM of the faster motion is independent of Q. Figure 1 shows HWHMs associated with internal and Brownian dynamics for 2 M NALMA at 281 K and T_R, as inferred from low-resolution data.

The Q independence of the HWHM of the faster dynamical component suggests that the corresponding dynamics is strongly localized. Considering the short length and time scales, this suggests rotational types of motion involving the methyl groups, which represent 75% of the total protons. The side-chain and backbone dynamics are dominated by methyl-group librations and rotations when there is only one hydration layer. Methyl-group rotations can be approximated with the three-site jump reorientation model [10]. The corresponding residence time between methyl flips is of the order of 12 ps at 281 K and 4.5 ps at T_R. The estimated activation energy, related to the height of the potential barrier hindering rotational motion, has an average value of 8.7 kJ/mol [1,11]. Activation energies depend on the type of amino-acid residue and on the residue's environment. Figure 2 shows the measured elastic incoherent structure factors (EISFs) [10], together with the results of fits based on the three-site jump reorientation model, from which we obtain the fraction of protons involved and the radius of the jump circle. This radius is roughly 1 Å, and the fraction of protons involved in the detected motions varies from about 57% at 281 K to 65% at T_R. Important information is thus emerging from this dynamical picture. A more relaxed water network, which can be attained with several hydration shells, is necessary to allow a hydrophobic side chain to exhibit the full range of motions characteristic of a protein above the dynamical transition temperature. The structure and the highly suppressed dynamics of the first hydration layer [5] strongly affect the interfacing solute dynamics, constraining the explored space and (of most importance) suppressing translational diffusive motion.

In order to investigate the solvent-driven dynamics in greater depth and to confirm previous results and speculations, we used the elastic scattering experiment to probe the MSFs of the protons. To avoid freezing the samples we restricted the measurements to temperatures above the dynamical transition temperature. The NALMA and NAGMA systems were both studied at the concentration that corresponds to one hydration layer of solvent (2$M$and 3M, respectively), and the results were analyzed in the Gaussian approximation [9]. In both cases plots of $\ln[I(Q)]$ versus Q^2 (not shown) have distinctly different slopes in the lower and higher ranges of Q. Corresponding MSFs, determined from the slopes, are shown in Fig. 3. One of the MSFs, designated $\langle u^2\rangle_1$ and determined in the Q range 0.4–1.4 Å^{-1}, is temperature dependent; the other one, designated $\langle u^2\rangle_2$ and obtained in the Q range 1.4–2.4 Å^{-1}, is independent of temperature.

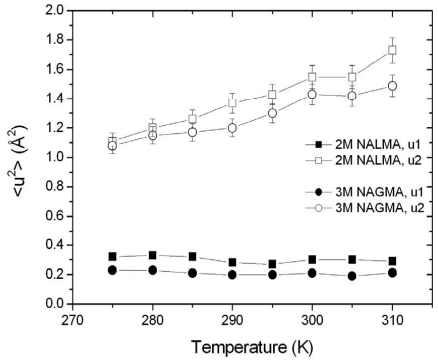

FIG. 3. Mean-square fluctuations $\langle u^2\rangle_1$ and $\langle u^2\rangle_2$ (labeled by $u1$ and $u2$) plotted as a function of temperature for 2 M NALMA and 3 M NAGMA. Open symbols represent mean-square displacements associated with the global dynamics. Solid symbols represent mean-square proton fluctuations associated with the internal dynamics. Error bars are smaller than the symbols used.

The range of amplitudes of the T-dependent MSF $\langle u^2\rangle_1$ and the Q range of the fit suggest that these fluctuations are associated with the space explored in the experimental time scale during random walk diffusion of the molecule. The T-independent MSF $\langle u^2\rangle_2$ may be associated with internal fluctuations. The lack of any dependence on T of the internal MSF confirms that the strongly suppressed hydration water dynamics, even at T_R [5], only allows methyl-group rotational motions and that diffusive kinds of motions are not experienced in this hydration solute configuration. The caging effect of water around hydrophobic groups lowers the mobility so that rotational motions, which involve smaller volume changes than translations, are allowed.

Powder-hydrated proteins ($h \sim 0.6$ g/g) show a nonlinear T dependence of the proton MSFs [4] whereas linear dependence is observed in completely dehydrated powders ($h \sim 0$). The same trend has been associated with hydrated protein methyl groups (0.6–4.5 g/g) [2]. These results attest to the crucial role of water, but they do not reveal the molecular mechanism of the solvent motion needed to enable protein structural relaxation. In fact these studies yield average information over the whole protein, it being impossible to distinguish between hydrophobic and hydrophilic parts and regions more or less exposed to the solvent. In addition, these experiments were performed on hydrated powders or on low-concentration solutions.

Our contribution to this topic has been to perform a *local measurement* of the influence of water dynamics on the side-chain dynamics of a small hydrophobic solute. Working at a hydration level of ~2.4 g/g, in contrast to what is observed in complete proteins, we do not observe any T dependence of the MSF in the investigated T range. We have only observed confined rotational motions consequent upon the structural and dynamical properties of the specific interfacial water network. Therefore, we support the argument that the structural and dynamical properties of the interfacial water strongly influence the side-chain dynamics and the activation of diffusive motion. We emphasize that the supercooled dynamical properties of surface water in the liquid state are intimately connected to the ability of water molecules to rearrange with other molecules, primarily across the peptide or protein surface, preventing relaxation of the water network and an increase in peptide or protein mobility [4].

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[1] J. E. Roh, V. N. Novikov, R. B. Gregory, J. E. Curtis, Z. Chowdhuri, and A. P. Sokolov, Phys. Rev. Lett. **95**, 038101 (2005).

[2] J. E. Curtis, M. Tarek, and D. J. Tobias, J. Am. Chem. Soc. **126**, 15928 (2004).

[3] W. Doster and M. Settles, Biochim. Biophys. Acta **1749**, 173 (2005).

[4] D. Russo, P. Baglioni, E. Peroni, and J. Teixeira, Chem. Phys. **292**, 235 (2003); M. Tarek and D. J. Tobias, Biophys. J. **79**, 3244 (2000); P. Kumar, Z. Yan, L. Xu, M. G. Mazza, S. V. Buldyrev, S. H. Chen, S. Sastry, and H. E. Stanley, Phys. Rev. Lett. **97**, 177802 (2006); A. Oleinikova, N. Smolin, and I. Brovchenko, J. Phys. Chem. B **110**, 19619 (2006); V. M. Dadarlat and C. B. Post, Biophys. J. **91**, 4544 (2006); T. M. Raschke, Curr. Opin. Struct. Biol. **16**, 152 (2006); B. Halle, Philos. Trans. R. Soc. London, Ser. B **359**, 1207 (2004); J. C. Smith, F. Merzel, A. N. Bondar, A. Tournier, and S. Fischer, *ibid.* **359**, 1181 (2004); M. Tarek and D. J. Tobias, Phys. Rev. Lett. **89**, 275501 (2002); M. C. Bellissent-Funel, J. Mol. Liq. **84**, 39 (2000); W. Doster, S. Cusack, and W. Petry, Nature (London) **337**, 754 (1989).

[5] D. Russo, G. Hura, and T. Head-Gordon, Biophys. J. **86**, 1852 (2004); D. Russo, R. K. Murarka, G. Hura, E. Verschell, J. R. D. Copley, and T. Head-Gordon, J. Phys. Chem. B **108**, 19885 (2004); D. Russo, R. K. Murarka, J. R. D. Copley, and T. Head-Gordon, *ibid.* **109**, 12966 (2005); G. Hura *et al.*, Perspect. Drug Discovery Des. **17**, 97 (1999).

[6] J. R. D. Copley and J. C. Cook, Chem. Phys. **292**, 477 (2003).

[7] F. Natali, D. Russo, M. Tehei, M. Bée, and A. Deriu, in *Quasi-Elastic Neutron Scattering Conference 2006 (QENS2006)*, edited by P. E. Sokol, H. Kaiser, D. Baxter, R. Pynn, D. Bossev, and M. Leuschner, (Materials Research Society, Warrendale, PA, 2007), p. 183.

[8] http://www.ncnr.nist.gov/dave

[9] F. Gabel, Eur. Biophys. J. **34**, 1 (2005).

[10] M. Bée, *Quasi-elastic Neutron Scattering* (Hilger, Philadelphia, 1988).

[11] B. Frick and L. J. Fetters, Macromolecules **27**, 974 (1994).