Collective dynamics of hydrated β-lactoglobulin by inelastic x-ray scattering

ARTICLE *in* THE JOURNAL OF CHEMICAL PHYSICS · OCTOBER 2010

Impact Factor: 2.95 · DOI: 10.1063/1.3484238 · Source: PubMed

4 AUTHORS, INCLUDING:

Koji Yoshida
Fukuoka University
62 PUBLICATIONS **682** CITATIONS
SEE PROFILE

Alfred Q.R. Baron
Japan Synchrotron Radiation Research Instit…
260 PUBLICATIONS **2,319** CITATIONS
SEE PROFILE

Toshio Yamaguchi
Fukuoka University
203 PUBLICATIONS **3,783** CITATIONS
SEE PROFILE

Collective dynamics of hydrated β-lactoglobulin by inelastic x-ray scattering

Koji Yoshida,[1,a)] Shinya Hosokawa,[2] Alfred Q. R. Baron,[3] and Toshio Yamaguchi[1]
[1]*Department of Chemistry, Fukuoka University, 8-19-1 Nanakuma, Jonan-ku, 814-0180 Fukuoka, Japan*
[2]*Center for Materials Science Using Third-Generation Synchrotron Radiation Facilities, Hiroshima Institute of Technology, 2-1-1 Miyake, Saeki-ku, Hiroshima 731-5193, Japan*
[3]*Materials Dynamics Laboratory, RIKEN SPring-8 Center, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo 679-5148, Japan and Research and Utilization Division, SPring-8/JASRI, 1-1-1 Kouto, Sayo-cho, Sayo-gun, Hyogo 679-5198, Japan*

(Received 11 February 2010; accepted 10 August 2010; published online 4 October 2010)

Inelastic x-ray scattering measurements of hydrated β-lactoglobulin (β-lg) were performed to investigate the collective dynamics of hydration water and hydrated protein on a picosecond time scale. Samples with different hydration levels h [=mass of water (g)/mass of protein (g)] of 0 (dry), 0.5, and 1.0 were measured at ambient temperature. The observed dynamical structure factor $S(Q,\omega)/S(Q)$ was analyzed by a model composed of a Lorentzian for the central peak and a damped harmonic oscillator (DHO) for the side peak. The dispersion relation between the excitation energy in the DHO model and the momentum transfer Q was obtained for the hydrated β-lg at both hydration levels, but no DHO excitation was found for the dry β-lg. The high-frequency sound velocity was similar to that previously observed in pure water. The ratio of the high-frequency sound velocity of hydrated β-lg to the adiabatic one of hydrated lysozyme ($h=0.41$) was estimated as ~1.6 for $h=0.5$. The value is significantly smaller than that (~2) of pure water that has the tetrahedral network structure. The present finding thus suggests that the tetrahedral network structure of water around the β-lg is partially disrupted by the perturbation from protein surface. These results are consistent with those reported from Brillouin neutron spectroscopy and molecular dynamics simulation studies of hydrated ribonuclease A. © *2010 American Institute of Physics*.
[doi:10.1063/1.3484238]

I. INTRODUCTION

Protein hydration plays a fundamental role in protein behavior: water-protein interactions affect protein folding, maintain structural integrity, mediate molecular recognition, and accelerate enzymatic catalysis. Many scattering measurements, both elastic and inelastic, and many molecular dynamics simulations, have been performed to investigate the relation between protein dynamics and that of the surrounding solvent molecules.[1] *Single particle* dynamics of proteins and hydrated water have been investigated using incoherent scattering from hydrogen in hydrated protein powder. The dynamical features of the hydration water of proteins with $h>0.2$ (Ref. 2) are seen to be considerably different from that of bulk water: the hydration water of proteins behaves like it is in a glassy state, characterized by a boson peak[3–6] at low-frequency and strong slowing-down of the diffusional motion.[7–11] The boson peak is sensitive to the degree of hydration and has been interpreted in terms of a water-coupled side-chain libration.[3] Recent molecular dynamics simulation studies by Joti *et al.*[12–14] suggested that the origin of the boson peak is due to the drastic change in the energy landscape of proteins by hydration.

The *collective* dynamics of hydrated protein may be investigated by light scattering and coherent inelastic x-ray and neutron scattering. These methods provide dynamical information based on correlation between different molecules and, for x-rays and neutrons, reflect the short wavelength density fluctuations and the sound propagation on a picosecond time scale.[15] The wavelength used in light scattering is some 10^3 Å, much longer than the atomic separation in proteins and water. Consequently, the light scattering data probe the dynamics of the sound propagation in a mesoscopic range and in a nanosecond time scale; the data are often interpreted in terms of a hydrodynamic theory.[16–18] In fact, the slope in the dispersion relation between the momentum transfer and the excitation energy corresponds to the adiabatic sound velocity. In contrast, neutrons and x-rays are suitable probes to investigate the microscopic region in a picosecond time scale because their wavelengths are comparable with the atomic distances of proteins and water.

The first observation of a collective excitation in water was made by neutron scattering.[19] Many inelastic x-ray scattering (IXS) experiments were performed to reveal the collective dynamics of pure water.[20–30] From the dispersion relation between the momentum transfer and the excitation peak energy, $\Omega(Q)$, the high-frequency sound velocity, $c_Q=\Omega(Q)/Q$, was found to be about twice the adiabatic sound velocity, reflecting the tetrahedrallike structure of water.[25,27,28] Thus, the collective dynamics measured by x-ray and neutron scattering contains more structural information than the single particle dynamics. X-ray scattering has an advantage over neutron with respect to the energy scan range. In neutron scattering, to detect the scattering at

a)Author to whom correspondence should be addressed. Electronic mail: kyoshida@fukuoka-u.ac.jp. FAX: +81-92-865-6030.

low Q, the energy scan range is usually limited by the low-energy of the neutrons. For example, in Brillouin neutron scattering (BNS) recently performed, the energy range was limited to ± 10 meV at Q of 4 nm^{-1}.[31] On the other hand, in IXS experiments, the energy scan range is much wider, e.g., ± 30 meV as in the present study, which improves accuracy in a fitting process of the dynamical structure factor.

Tarek *et al.*[32] reported the results of molecular dynamics (MD) simulation of the collective dynamics of water in a hydrated protein crystal at 150 and 300 K. They predicted the existence of two sound modes in the hydrated protein: a highly dispersive mode around 35 meV and a weakly dispersive one around 9 meV in the Q range studied ($5 < Q < 42$ nm^{-1}). It should be noted that the structure and dynamics of protein depend on the interatomic potential used in MD calculations as claimed by Yoda *et al.*[33] They investigated the secondary structural characteristics of polypeptides using the force fields for protein systems developed by different research groups. Therefore, experimental information on collective dynamics of hydrated proteins is very important in verification of simulation results. Neutron spin echo[15,34–36] and IXS experiments[37,38] have been used to investigate the collective dynamics of lipid bilayers in a nano- and a picosecond time scale, respectively. However, there are few experimental studies on collective dynamics of protein in a picosecond time scale. The pioneer work was the BNS measurement of dry and hydrated C-phycocyanin by Bellissent-Fune l *et al.*,[39] followed by a BNS study of hydrated ribonuclease A (RNase A),[31] and IXS experiments of hydrated bovine serum albumin[40] (BSA) and lysozyme.[40,41] In the IXS study on hydrated BSA, no linear dispersion relation was observed between the momentum transfer and the excitation peak in a low Q region < 10 nm^{-1}.[40] In contrast, the linear dispersion relation between ω and the excitation peak reported in the BNS experiment on hydrated RNase A (Ref. 31) is consistent with the MD results[32] where the energy range observed was limited to ± 10 meV at Q of 4 nm^{-1}. Thus, to verify the MD simulation and to clarify how the collective dynamics of hydration water changes for various proteins, more IXS experiments are needed over a wider energy range for hydrated proteins with different structures.

In the present study, we have chosen β-lactoglobulin (β-lg) (PDB ID: 1BEB), that is, a dimer of about 36 kDa molecular weight, each of which consists of nine antiparallel β-sheets and one α-helix segment.[42,43] The thiol-disulfide exchange in it is the origin of the monomer-dimer transformation, and the protein molecules easily aggregate each other and gelate by heat[44] and cosolvent[45,46] in contrast with a single globular protein, such as lysozyme. Thus, it should be noted that the secondary structure and the interaction between β-lg and water are very different from those of RNase,[31] BSA,[40] and lysozyme[40,41] previously studied. IXS of hydrated β-lg was measured at room temperature at $h = 0.5$ (water adsorbed powder) and 1.0 (solution). From the present results, discussion will be made on the followings: how the collective dynamics of protein hydration water in a picosecond time scale differs from that of bulk water, a comparison of high-frequency sound propagation with other water dynamics, and to which extent the high-frequency sound velocity deviates from the corresponding adiabatic sound velocity of a hydrated protein. Finally, the present results for hydrated β-lg will be compared with those for other hydrated proteins with different secondary structures to see whether or not the collective dynamics depends on the secondary structure.

II. EXPERIMENTAL

A. Samples

The powdery mixture of gene variants, β-lg A and β-lg B (PDB ID is 1BEB), from bovine milk, 90% PAGE (L0130, lot 095K7006), was purchased from SIGMA-ALDRICH and used without further purification. The powder was dried with a turbomolecular pump for 1 day. Three β-lg samples were measured: water adsorbed β-lg powder at $h = 0.5$ and β-lg-water mixture at $h = 1.0$ as well as dry β-lg ($h = 0$). The water absorbed β-lg sample was prepared as follows: the dry powder was kept in a desiccator filled with saturated water vapor until h reached 0.5. The β-lg-water mixture was prepared by mixing dry β-lg powder and distilled water of the same mass. The dry protein itself contains some amounts of crystallization water, which is not taken into account in h in this study as in other neutron scattering measurements of hydrated proteins at various hydration levels.[2,9,31]

A sample cell was made of a single crystal sapphire because of its low background in a low Q region and its very high sound velocity ($> 10\,000$ ms^{-1}). Thus, the signal of a sample is not disturbed by that of the cell.[47] A sapphire tube of one end closed with inner and outer diameters of 2.5 and 3.5 mm, respectively, was inserted into a similar type of larger tube with inner and outer diameters of 3.6 and 4.5 mm, respectively. The closed ends act as x-ray windows whose thickness was grinded to about 0.2 mm each. Samples were kept in the space between the two windows. The thickness of sample was adjusted to be 5 mm, and then the two tubes were glued with an epoxy adhesive to seal the wet samples. The sample cell was kept in a chamber that has single crystal Si windows under helium atmosphere of about 1 bar to suppress the air scattering effect.

B. IXS measurements

The IXS experiments were performed on a high-resolution spectrometer installed at the beamline BL35XU of the SPring-8.[48] A highly resolved monochromatized x-ray beam of 4×10^9 photons s^{-1} was obtained from cryogenically cooled Si(111) double-crystals followed by a Si(11 11 11) monochromator operating in an extreme backscattering geometry ($\sim 89.98°$, 21.75 keV). The scattered x-ray photons were acquired by 12 spherically curved Si analyzers with a diameter of 10 cm using the same backscattering geometry and counted by CdZnTe detectors. Energy scans were made at each scattering angle by thermal variation of the lattice parameter of the monochromator crystal. The energy resolution of the spectrometer was determined from the scattering of a Plexiglas with the same thickness of the protein sample, and the overall energy resolution was 1.43–1.82 meV full width at half maximum. The Q resolution was

± 0.48 nm^{-1}, where $Q=4\pi/\lambda \sin\theta, 2\theta$ is the scattering angle and λ is the wavelength of x-rays. IXS experiments were carried out at the Q values from 2 to 17 nm^{-1} to cover the Q-range within which the first and the second peaks of the structure factor $S(Q)$ for the dry protein fall. A typical energy scan ranging ± 30 meV took approximately 2 h. Two or three scans were performed to improve the counting statistics.

C. Data analysis

The IXS spectra of samples were corrected for absorption and an empty cell. Then, they were normalized by the static structure factor $S(Q)$ obtained by integration of $S(Q,\omega)$ over an energy range measured at individual Q values. The IXS spectra can be expressed as $I(Q,\omega)=S(Q,\omega) \otimes R(Q,\omega)$, where $S(Q,\omega)$ and $R(Q,\omega)$ are the dynamical structure factor and the instrumental resolution function, respectively, and the symbol $\otimes$ means numerical convolution.

To analyze $S(Q,\omega)$, we employed the following function which consists of a Lorentzian for the central elastic peak and two damped harmonic oscillators (DHOs) for both side peaks,[49]

$$S(Q,\omega) = \left[\frac{\hbar\omega/k_BT}{1-\exp(-\hbar\omega/k_BT)}\right]\frac{A_0}{\pi}\frac{\Gamma_0}{\Gamma_0^2+\omega^2} + \left[\frac{1}{1-\exp(-\hbar\omega/k_BT)}\right] \times \frac{A(Q)}{\pi}\frac{4\omega\Gamma(Q)\sqrt{\Omega(Q)^2-\Gamma(Q)^2}}{(\omega^2-\Omega(Q)^2)^2+4\omega^2\Gamma(Q)^2}, \quad (1)$$

where A_0 and Γ_0 are the intensity and width of a central peak, respectively. $\hbar$, k_B, and T are the Plank constant, the Boltzmann constant, and temperature, respectively. $A(Q)$ and $\Gamma(Q)$ are the intensity and width (damping factor) of the inelastic peaks, respectively, at positions $\pm\Omega(Q)$ of an excitation peak due to inelastic scattering. The factor, $\hbar\omega/k_BT(1-\exp(-\hbar\omega/k_BT))^{-1}$, denotes the Bose factor, asymmetrical weight factor for Stokes, and anti-Stokes regions, which is a function of temperature.

III. RESULTS AND DISCUSSION

A. IXS spectra

Figure 1(a) shows typical normalized IXS spectra of β-lg at $h=0.5$ and 1.0 as a function of excitation energy ω at $Q=6.1$ nm^{-1}. Those of dry β-lg and the resolution function are also depicted for comparison in Fig. 1(a). The IXS spectrum of the dry sample is similar to the resolution function, showing no dispersion present for the dry protein. Two inelastic excitation peaks are discernible at around ± 12 meV in both hydrated samples, although they seem less significant for $h=0.5$. These spectra were fitted with the convolution function of the DHO model of Eq. (1) and the resolution function. The fitting results are shown in solid lines in Fig. 1(a). Figures 1(b) and 1(c) show the observed and fitted excitation peaks as well as the DHO components enlarged by 10^3 at $h=0.5$ and 1.0, respectively.

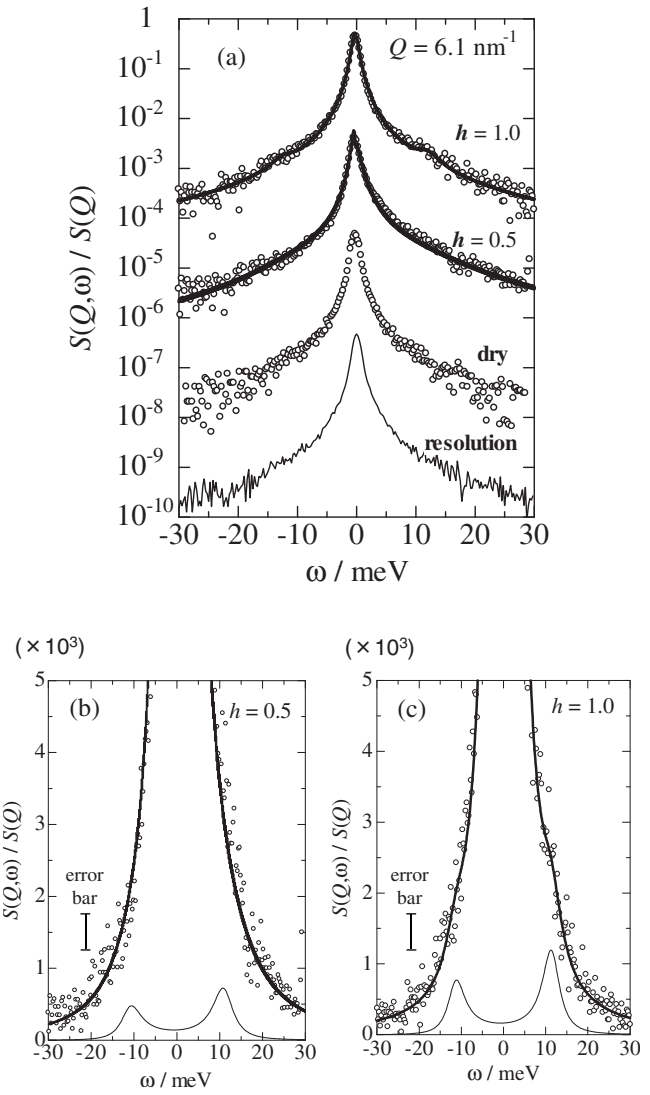

FIG. 1. (a) The IXS spectra of dry and hydrated β-lg at $h=0.5$ and 1.0 normalized by the structure factor, $S(Q,\omega)/S(Q)$, as a function of excitation energy ω at $Q=6.1$ nm^{-1}. The solid line indicates the resolution function and the thick solid lines the DHO fitting results. (b) The enlarged view of the IXS spectra of hydrated β-lg at $h=0.5$. (c) The enlarged view of the IXS spectra of hydrated β-lg at $h=1.0$. In these two figures, the thick solid lines indicate the DHO fitting results and the thin solid lines the DHO component in Eq. (1).

B. Static structure factor

Figure 2 shows $S(Q)$ values of the dry β-lg and the hydrated protein at $h=0.5$ and 1.0. The $S(Q)$ values were obtained from the integration of $S(Q,\omega)$ over an energy range measured at individual Q values, and thus the errors of $S(Q)$ estimated from those of $S(Q,\omega)$ were relatively large. There appear the first peak at ~ 7 nm^{-1} and the second one at ~ 13 nm^{-1} for all samples. Previously, similar peaks have been reported for other hydrated proteins.[40,50] For hydrated BSA and lysozyme,[40] the peak at ~ 7 nm^{-1} was assigned to a characteristic protein-protein interaction, whereas the peak at 13 nm^{-1} to the short-range ordering of the secondary structure, such as β-sheet whose distance is 4.5 Å and α-helices whose pitch is 5 Å. Very recently, a polarization analysis of neutron scattering data for hydrated myoglobin and C-phycocyanin has reported that the former peak is re-

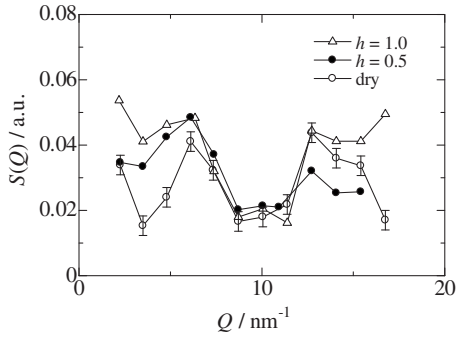

FIG. 2. The static structure factor $S(Q)$ of dry and hydrated β-lg at $h=0.5$ and 1.0. They were obtained by the integration of the dynamical structure factor $S(Q,\omega)$ with ω.

flecting the secondary structure, while the latter one is due to side-chain interference.[50] The present method cannot provide direct evidence to conclude the assignment of the peaks. However, a similar feature of the first peak at ~7 nm^{-1} for both samples could prefer the latter[50] to the former assignment[40] since the protein-protein interaction might differ at the different hydration levels.

When $h=1.0$, a sharp rise is observed at ~17 nm^{-1}, which is close to the first peak in $S(Q)$ of bulk water (19 nm^{-1}).[51] The corresponding phenomenon has not been reported in $S(Q)$ of hydrated BSA and lysozyme at $h=0.3$.[40] In addition, it is at $h=0.345$ that water molecules fully cover the surface of β-lg.[52] Considering all these findings, the peak at ~17 nm^{-1} do suggest that a considerable amount of water is available in the second hydration shell of β-lg at $h=1.0$.

C. High-frequency sound velocity

Figure 3 shows the Q dependence of the position $\Omega(Q)$ of an excitation peak in Eq. (1) for hydrated β-lg of $h=0.5$ and 1.0. The $\Omega(Q)$ values of both hydrated β-lg are almost proportional to Q in the Q region from 2 to ~8 nm^{-1}. The high-frequency sound velocity c_Q, which is defined as $\Omega(Q)/Q$, was obtained by a least squares fitting procedure over a Q range of 2–8 nm^{-1}. The optimized values obtained were 2895 ± 160 and 2862 ± 110 m s^{-1} for the β-lg samples with $h=0.5$ and 1.0, respectively. Since the excitation mode is not observed for the dry β-lg, the present results show that protein dynamics of β-lg changes considerably by hydration as previously discussed in the literature.[2]

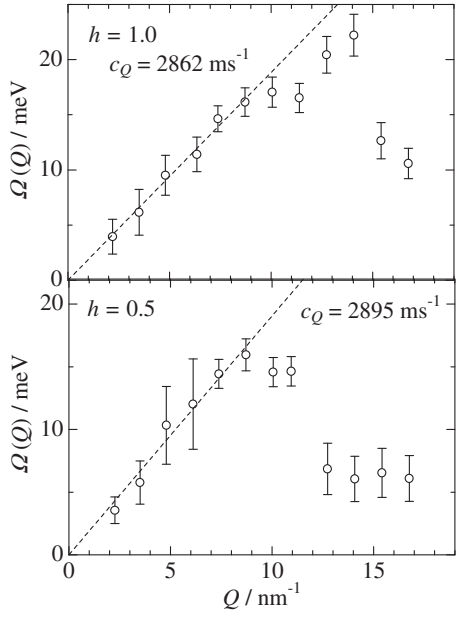

FIG. 3. The dispersion relation between the inelastic excitation energy $\Omega(Q)$ and the momentum transfer Q for hydrated β-lg at $h=0.5$ and 1.0. Two dashed lines show the least-squares fits in a low Q-region. The values of the high-frequency sound velocity c_Q were obtained from the slope of the fits.

The MD simulation[32] reported that the high-frequency sound velocity of hydrated water in RNase is 3800 m s^{-1} at $h=0.5$, considerably larger than the present value. On the other hand, the present value is close to that (3200 ± 150 m s^{-1}) for heavy water absorbed in RNase at $h=0.68$ from the BNS measurements[31] and that (3300 m s^{-1}) of heavy water absorbed C-phycocyanin at $h=0.5$.[39] In the BNS measurements, the high-frequency sound velocities showed a somewhat increase in the sound speed with increasing water content; i.e., 3200 ± 150 m/s at $h=0.68$ and 3520 ± 100 m/s at $h=1.00$. In the present study, however, such an increase in the sound velocity was not observed within the experimental certainties. The coherent signals in the BNS measurements are mostly contributed from deuteron in heavy water, whereas the IXS study observes the collective dynamics of not only hydration water but also protein itself. The similarity of the high-frequency sound velocity between the IXS and the BNS measurements does suggest that the contribution of hydration water is more dominant than that of the protein in the high-frequency sound propagation obtained by the present IXS.

Here, it should be noted that the BNS (Ref. 31) and MD (Ref. 32) studies on hydrated RNase reported a low-frequency nondispersive mode at constant energies of 6–7 and ~10 meV, respectively. Attempts to fit the present data with two DHO modes were not successful because of the broadening of the central peak due probably to the motion of the protein. As seen in Fig. 1, however, the observed dynamical structure factors were satisfactorily reproduced with one DHO mode. A single DHO mode has also been reported in the IXS measurements of hydrated BSA.[40]

In Fig. 3, the $\Omega(Q)$ values seem to approach a minimum at 12–17 nm^{-1}, within which the first maximum in $S(Q)$ falls, for the samples of $h=0.5$ and 1.0. A similar result is also seen in liquids, such as monoatomic liquids[53] and methanol.[54]

Next, the high-frequency sound velocity of the hydrated β-lg was compared with the adiabatic sound velocity of the corresponding system to discuss the microscopic structure of hydration water of β-lg. For pure water, the ratio of the high-frequency sound velocity to the adiabatic one is ~2 in ambient condition and decreases with increasing of pressure[25] and decreasing of density.[27,28] This result has been interpreted as the weakening of tetrahedral network structure of water.[25,27,28] To our knowledge, there is no adiabatic sound velocity data available for hydrated β-lg. However, the adiabatic sound velocity of hydrated lysozyme crystal at $h=0.41$ was reported to be 1817 m s^{-1}.[55] Using this value in place of that of the hydrated β-lg, we estimated a ratio of the

high-frequency sound velocity of β-lg to the adiabatic one as ~ 1.6 at $h=0.5$. The value is significantly smaller than that (~ 2) of pure water that has the tetrahedral network structure.[56,57] The present finding thus suggests that the tetrahedral network structure of water around β-lg is weakened even in the second hydration shell by the perturbation from the protein surface.

D. Comparison of high-frequency sound propagation with other dynamics of hydrated proteins

The high-frequency sound propagation of hydrated β-lg from the present IXS measurements was 2895 ± 160 ms^{-1}, not very different from the value of pure water [3200 ± 100 ms^{-1} (Ref. 20)]. The present value is in good agreement with that obtained on hydrated RNase by BNS.[31]

This dynamical information in terms of the high-frequency sound propagation is in marked contrast with the MD, NMR, and quasi-elastic neutron scattering (QENS) results on hydrated RNase,[7] ubiquitin,[8] C-phycocyanin,[9] and lysozyme,[10] i.e., the other methods showed that the translational and rotational motions of hydrated water of the proteins is slower by two order magnitude than that of bulk water. This apparent inconsistency between the IXS and MD, NMR, and QENS results would be due to the fact that IXS observes the different motions of water molecule in different time scales from those seen with MD, NMR, and QENS. The time scales observable by MD (Ref. 7) and NMR (Ref. 8) are in a few picoseconds. The excitation energy probed by QENS is in the order of sub-meV that again corresponds to the time scale of a few picoseconds. Therefore, dynamical information on the translational and rotational motions of water molecules[9,10] given by MD, NMR, and QENS is relevant to the forming and breaking of hydrogen bonds between water molecules. On the other hand, the excitation energy detected by IXS is in a few meV that corresponds to the time scale of a few hundred femtoseconds. Thus, the high-frequency sound propagation investigated by IXS would reflect the fluctuations of water molecules around an equilibrium distance of the hydrogen bond, which is related to the thermal vibrations represented as the Debye–Waller factor in the form of $\exp(-Q^2\langle U(0)^2\rangle/3)$, where $U(0)$ is the displacement of the center of water molecule at a time $t=0$. The similarity of the high-frequency sound velocity between hydrated β-lg and pure water suggests that the fluctuations of water molecules in the protein are not very different from those in pure water. On the other hand, the translational and rotational diffusions of water molecules accompany the breaking and forming of hydrogen bonds between water molecules in bulk and between water molecules and protein surface groups for hydrated proteins. On the basis of the present findings, we could say that the potential barrier for protein hydration water to form and break the hydrogen bonds is higher than that for pure water, whereas the thermal vibrations of water molecules are in the same order in protein hydration water and in bulk.

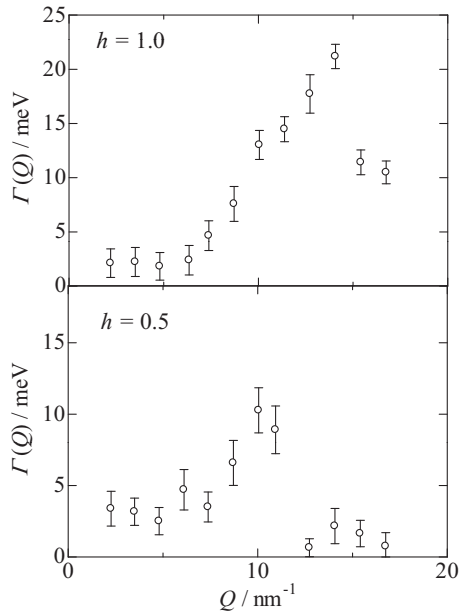

FIG. 4. The damping factor $\Gamma(Q)$ of the DHO function for the hydrated β-lg at $h=0.5$ and 1.0.

E. Damping factor

According to the hydrodynamic theory of a continuous model, the damping factor of sound waves to propagate in simple liquids shows the Q^2-dependence, as found in light scattering experiments on liquid argon.[58] This might not be the case for higher frequency dynamics in a microscopic region observed by the present IXS study. In fact, the damping factor for pure water from IXS measurements[20] was constant at $Q<10$ nm^{-1} and then increased linearly with Q. In Fig. 4, we showed the Q-plot of the damping factor for the hydrated β-lg at $h=0.5$ and 1.0. The $\Gamma(Q)$ values at $h=0.5$ and 1.0 are both almost constant in a Q-region from ~ 2 to 7–8 nm^{-1} and then increase linearly with Q over a Q-range of 7–10 and 8–14 nm^{-1}, respectively. Similar results are reported for hydrated BSA by IXS, i.e., the damping factor is almost constant at $Q<\sim 10$ nm^{-1}.[40] The linewidth of hydrated C-phycocyanin from BNS measurements also appeared constant at $Q<4$ nm^{-1}.[39] The origin of the plateau in the damping factor in the low Q-region is not clear yet. Furthermore, the linear relationship of the damping factor with Q from the present IXS experiments is not consistent with the results from the BNS measurements on hydrated RNase; the latter showed the Q^2-dependence in the Q region ≤ 15 nm^{-1}. Thus, further experiments by IXS and BNS would be needed to draw a definite conclusion of damping factor of hydrated proteins. At $Q>10$ and 14 nm^{-1} for $h=0.5$ and 1.5, where the first peak of $S(Q)$ of hydrated β-lg appears, the $\Gamma(Q)$ values decreased drastically, which is called as "de Gennes narrowing."[59]

F. Comparison with other proteins

The present results on $h=0.5$ and $h=1.0$ solutions of β-lg show that the acoustic mode frequency increases linearly with Q for $2<Q<8$ nm^{-1}. Similar results have been reported for hydrated lysozyme by BNS (Ref. 31) and MD

simulation.[32] Results by Shiotani *et al.*[41] showed similar behavior for lysozyme solutions with $h > \sim 2$, and that the acoustic mode became harder to see as h decreased from ~ 1000 to ~ 2. Meanwhile Liu *et al.*[40] found no acoustic mode for lysozyme with $h \sim 0.3$. These results are all reasonably consistent with a model of decreasing fractional contribution of the acoustic mode to the scattering as the hydration is reduced, though additional IXS measurements would help to clarify the detailed dependence on protein and hydration level.

IV. CONCLUSIONS

Collective dynamics of hydrated β-lg at $h=0.5$ and 1.0 was measured in a Q-range between 2–17 nm^{-1} with IXS at ambient temperature. The high-frequency sound velocity obtained from the dispersion relation between phone excitation energy and Q was similar to that of bulk water. This finding is inconsistent with the results reported on hydrated water of RNase,[7] Ubiquitin,[8] C-phycocyanin,[9] and lysozyme[10] by MD, NMR, and QENS. This fact suggests that the collective dynamics from IXS would reflect the atomic vibration of water molecules around an equilibrium distance, whereas the single particle dynamics from QENS is related to the forming and breaking of hydrogen bonds between water molecules. The ratio of the high-frequency sound velocity of hydrated β-lg to the adiabatic one of hydrated lysozyme at $h=0.41$ was estimated as ~ 1.6 for $h=0.5$. These values are significantly smaller than that (~ 2) of pure water that has a tetrahedral network structure. This finding suggests that the structure of protein hydrated water is more disordered than that of the bulk. These results are consistent with those of hydrated ribonuclease powder from BNS experiments and MD simulation although IXS investigates dynamics from both protein and hydration water. Therefore, hydration water in hydrated β-lg could mainly contribute to the IXS spectra.

ACKNOWLEDGMENTS

The authors thank Professor Masanori Inui and Dr. Yukio Kajihara in Hiroshima University for assisting in manufacturing of the sapphire cell. The experiment was performed with BL35XU in the SPring-8 with the approval of the Japan Synchrotron Radiation Research Institute (JASRI) (Proposal No. 2008A1491). This research was partially supported by the Ministry of Education, Science, Sports and Culture, Grant-in-Aid for Scientific Research on Priority Areas, Grant No. 70158111, 2003–2007, and Young Scientists (B), Grant No. 19750010, 2007–2009.

[1] *Hydration Processes in Biology*, M.-C. Bellissent-Funel (NATO Advanced Study Institute, Les Houches, 1988), references cited therein.
[2] W. Doster and M. Settles, Biochim. Biophys. Acta **1749**, 173 (2005).
[3] M. Diehl, W. Doster, W. Petry, and H. Schober, Biophys. J. **73**, 2726 (1997).
[4] A. Paciaroni, A. R. Bizzarri, and S. Cannistraro, Phys. Rev. E **57**, R6277 (1998).
[5] A. Paciaroni, A. R. Bizzarri, and S. Cannistraro, Phys. Rev. E **60**, R2476 (1999).
[6] H. Leyser, W. Doster, and M. Diehl, Phys. Rev. Lett. **82**, 2987 (1999).
[7] M. Tarek and D. J. Tobias, Biophys. J. **79**, 3244 (2000).
[8] V. P. Denisov and B. Halle, J. Mol. Biol. **245**, 698 (1995).
[9] M. C. Bellissent-Funel, J. M. Zanotti, and S.-H. Chen, Faraday Discuss. **103**, 281 (1996).
[10] F. Pizzitutti, M. Marchi, F. Sterpone, and P. J. Rossky, J. Phys. Chem. B **111**, 7584 (2007).
[11] M. Settles and W. Doster, Faraday Discuss. **103**, 269 (1996).
[12] Y. Joti, A. Kitao, and N. Go, J. Am. Chem. Soc. **127**, 8705 (2005).
[13] Y. Joti, H. Nakagawa, M. Kataoka, and A. Kitao, J. Phys. Chem. B **112**, 3522 (2008).
[14] Y. Joti, H. Nakagawa, M. Kataoka, and A. Kitao, Biophys. J. **94**, 4435 (2008).
[15] M. C. Rheinstädter, Biointerphases **3**, FB83 (2008).
[16] U. Balucani and M. Zoppi, *Dynamics of the Liquid State* (Oxford University Press, Oxford, 1994).
[17] P. A. Fleury and J. P. Boon, Phys. Rev. **186**, 244 (1969).
[18] P. A. Fleury and J. P. Boon, Adv. Chem. Phys. **24**, 1 (1973).
[19] J. Teixeira, M. C. Bellissent-Funel, S. H. Chen, and B. Dorner, Phys. Rev. Lett. **54**, 2681 (1985).
[20] F. Sette, G. Ruocco, M. Krisch, U. Bergmann, C. Masciovec-chio, V. Mazzacurati, G. Signorelli, and R. Verbeni, Phys. Rev. Lett. **75**, 850 (1995).
[21] F. Sette, G. Ruocco, M. Krisch, C. Masciovecchio, R. Verbeni, and U. Bergmann, Phys. Rev. Lett. **77**, 83 (1996).
[22] G. Ruocco and F. Sette, J. Phys.: Condens. Matter **11**, R259 (1999).
[23] A. Cunsolo, G. Ruocco, F. Sette, C. Masciovecchio, A. Mermet, G. Monaco, M. Sampoli, and R. Verbeni, Phys. Rev. Lett. **82**, 775 (1999).
[24] G. Monaco, A. Cunsolo, G. Ruocco, and F. Sette, Phys. Rev. E **60**, 5505 (1999).
[25] M. Krisch P. Loubeyre P. G. Ruocco, F. Sette, A. Cunsolo, M. D'Astuto, R. LeToullec, M. Lorenzen, A. Mermet, G. Monaco, and R. Verbeni, Phys. Rev. Lett. **89**, 125502 (2002).
[26] E. Pontecorvo, M. Krisch, A. Cunsolo, G. Monaco, A. Mermet, R. Verbeni, F. Sette, and G. Ruocco, Phys. Rev. E **71**, 011501 (2005).
[27] T. Yamaguchi, K. Yoshida, N. Yamamoto, S. Hosokawa, M. Inui, A. Q. R. Baron, and S. Tsutsui, Nucl. Instrum. Methods Phys. Res. B **238**, 146 (2005).
[28] T. Yamaguchi, K. Yoshida, N. Yamamoto, S. Hosokawa, M. Inui, A. Q. R. Baron, and S. Tsutsui, J. Phys. Chem. Solids **66**, 2246 (2005).
[29] F. Bencivenga, A. Cunsolo, M. Krisch, G. Monaco, G. Ruocco, and F. Sette, Phys. Rev. E **75**, 051202 (2007).
[30] F. Bencivenga, A. Cunsolo, A. M. Krisch, G. Monaco, G. Ruocco, and F. Sette, J. Chem. Phys. **130**, 064501 (2009).
[31] A. Orecchini, A. Paciaroni, A. De Francesco, C. Petrillo, and F. Sacchetti, J. Am. Chem. Soc. **131**, 4664 (2009).
[32] M. Tarek and D. J. Tobias, Phys. Rev. Lett. **89**, 275501 (2002).
[33] T. Yoda, Y. Sugita, and Y. Okamoto, Chem. Phys. Lett. **386**, 460 (2004).
[34] W. Pfeiffer, S. Konig, J. F. Legrand, T. Bayerl, D. Richter, and E. Sackmann, Europhys. Lett. **23**, 457 (1993).
[35] T. Takeda, Y. Kawabata, H. Seto, S. Komura, S. Gosh, M. Nagao, and D. Okuhara, J. Phys. Chem. Solids **60**, 1375 (1999).
[36] M. C. Rheinstädter, W. Häussler, and T. Salditt, Phys. Rev. Lett. **97**, 048103 (2006).
[37] S. H. Chen, C. Y. Liao, H. W. Huang, T. M. Weiss, M. C. Bellissent-Funel, and F. Sette, Phys. Rev. Lett. **86**, 740 (2001).
[38] T. M. Weiss, P.-J. Chen, H. Sinn, E. E. Alp, S.-H. Chen, and H. W. Huang, Biophys. J. **84**, 3767 (2003).
[39] M.-C. Bellissent-Funel, J. Teixeira, S. H. Chen, B. Dorner, H. D. Middendorf, and H. L. Crespi, Biophys. J. **56**, 713 (1989).
[40] D. Liu, X. Chu, M. Lagi, Y. Zhang, E. Fratini, P. Baglioni, A. Alatas, A. Said, E. Alp, and S.-H. Chen, Phys. Rev. Lett. **101**, 135501 (2008).
[41] N. Shiotani, N. Kamiya, A. Q. R. Baron, S. Tsutsui, and J. P. Sutter, SPring-8 User Experiment Report, No. 2005A0147-ND3d-np, 2005, Japan Synchrotron Radiation Research Institute (JASRI), 1-1-1, Kouto, Sayo-cho, Sayo-gun, Hyogo 679-5198 Japan.
[42] M. Z. Papiz, L. Sawyer, E. E. Eliopoulos, A. C. North, J. B. C. Findlay, R. Sivaprasadarao, T. A. Jones, M. E. New-comer, and P. J. Kraulis, Nature (London) **324**, 383 (1986).
[43] H. L. Monaco, G. Zanotti, P. Spadon, M. Bolognesi, L. Sawyer, and E. E. Eliopoulos, J. Mol. Biol. **197**, 695 (1987).
[44] S. Takata, T. Norisuye, N. Tanaka, and M. Shibayama, Macromolecules **33**, 5470 (2000).
[45] M. Barteri, M. C. Gaudiano, G. Mei, and N. Rosato, Biochim. Biophys. Acta **1383**, 317 (1998).
[46] K. Yoshida, T. Yamaguchi, N. Osaka, H. Endo, and M. Shibayama, Phys. Chem. Chem. Phys. **12**, 3260 (2010).
[47] S. Hosokawa, Condens. Matter Phys. **11**, 71 (2008).

[48] A. Q. R. Baron, Y. Tanaka, S. Goto, K. Takeshita, T. Matsushita, and T. Ishikawa, J. Phys. Chem. Solids **61**, 461 (2000).
[49] Y. Kawakita, S. Hosokawa, T. Enosaki, K. Ohshima, S. Takeda, W.-C. Pilgrim, S. Tsutsui, Y. Tanaka, and A. Q. R. Baron, J. Phys. Soc. Jpn. **72**, 1603 (2003).
[50] A. Gaspar, S. Busch, M. S. Appavou, W. Häusler, R. Georgii, Y. Su, and W. Doster, BBA Protein Proteomics **1804**, 76 (2010).
[51] A. H. Narten and H. A. Levy, J. Chem. Phys. **55**, 2263 (1971).
[52] K. Yoshida, T. Yamaguchi, M.-C. Belllissent-Funel, and S. Longeville, Eur. Phys. J. Spec. Top. **141**, 223 (2007).
[53] A. A. van Well, P. Verkerk, L. A. de Graaf, J.-B. Suck, and J. R. D. Copley, Phys. Rev. A **31**, 3391 (1985).
[54] K. Yoshida, N. Yamamoto, S. Hosokawa, A. Q. R. Baron, and T. Yamaguchi, Chem. Phys. Lett. **440**, 210 (2007).
[55] M. Tachibana, K. Kojima, R. Ikuyama, Y. Kobayashi, and M. Ataka, Chem. Phys. Lett. **332**, 259 (2000).
[56] U. Balucani, G. Ruocco, A. Torcini, and R. Vallauri, Phys. Rev. E **47**, 1677 (1993).
[57] F. Sciortino and S. Sastry, J. Chem. Phys. **100**, 3881 (1994).
[58] J. P. Boon and S. Yip, *Molecular Hydrodynamics* (McGraw-Hill, New York, 1980).
[59] P. G. de Gennes, Physica (Amsterdam) **25**, 825 (1959).