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Coupling of the hydration water dynamics and the internal dynamics of actin detected by quasielastic neutron scattering

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ABSTRACT

In order to characterize dynamics of water molecules around F-actin and G-actin, quasielastic neutron scattering experiments were performed on powder samples of F-actin and G-actin, hydrated either with D_2O or H_2O, at hydration ratios of 0.4 and 1.0. By combined analysis of the quasielastic neutron scattering spectra, the parameter values characterizing the dynamics of the water molecules in the first hydration layer and those of the water molecules outside of the first layer were obtained. The translational diffusion coefficients (D_T) of the hydration water in the first layer were found to be 1.2×10^{-5} cm^2/s and 1.7×10^{-5} cm^2/s for F-actin and G-actin, respectively, while that for bulk water was 2.8×10^{-5} cm^2/s. The residence times were 6.6 ps and 5.0 ps for F-actin and G-actin, respectively, while that for bulk water was 0.62 ps. These differences between F-actin and G-actin, indicating that the hydration water around G-actin is more mobile than that around F-actin, are in concert with the results of the internal dynamics of F-actin and G-actin, showing that G-actin fluctuates more rapidly than F-actin. This implies that the dynamics of the hydration water is coupled to the internal dynamics of the actin molecules. The D_T values of the water molecules outside of the first hydration layer were found to be similar to that of bulk water though the residence times are strongly affected by the first hydration layer. This supports the recent observation on intracellular water that shows bulk-like behavior.

1. Introduction

Proteins usually reside in an aqueous environment, and are fluctuating constantly under the influence of thermal fluctuations of surrounding solvent molecules, the most abundant of which is water molecules. It is now widely accepted that these thermal fluctuations, or dynamics, of proteins are essential for functions [1]. Ultimate understanding of the molecular mechanism of the protein function thus requires understanding of the dynamics of the protein as well as those of the surrounding water molecules.

In this context, we have been investigating the dynamics of the protein actin. F-actin, a filamentous polymer formed by polymerization of the monomers (G-actin), plays crucial roles in a variety of functions related to cell motility [2,3]. It is flexibility of the actin molecules that enables it to interact with various actin-binding proteins and thereby expressing various functions. Since flexibility arises from the dynamics, understanding the dynamics of actin is important for ultimate elucidation of the molecular mechanism of the multi-functions of actin. We employed neutron scattering for the measurements of the dynamics of actin. Neutron scattering provides a unique tool to directly measure the dynamics of the proteins [4]. We performed the elastic incoherent neutron scattering (EINS) and quasielastic neutron scattering (QENS) measurements on powder samples of F-actin and G-actin, hydrated with D_2O at the hydration ratios of 0.4 (g D_2O/g protein) and 1.0, which correspond to the samples containing only the first layer of hydration water and those containing more water than the first layer, respectively, and found the differences in the internal dynamics between F-actin and G-actin, such that G-actin fluctuates more rapidly than F-actin [5,6].

For full characterization of the dynamics of actin, however, it is important to know how such differences in the internal dynamics of actin are related to the dynamics of hydration water. Here, we employed QENS to extract the information on the dynamics of the hydration water around G-actin and F-actin. The QENS spectra of the powder samples of F-actin and G-actin, hydrated with D_2O or H_2O, at the hydration ratios of 0.4 and 1.0, were measured. Combined analysis of the QENS spectra of the powder samples hydrated with D_2O and H_2O provided the information on the dynamics of the hydration water.

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2. Materials and methods

2.1. Sample preparation

The hydrated powder samples of F-actin and G-actin were prepared from chicken breast muscle as described in the previous paper [6]. Hydration of the samples was done either with D_2O or H_2O. The hydration ratios (h) of the samples referred to as 0.4 and 1.0 were nominally 0.4 and 1.0, respectively.

2.2. Quasielastic neutron scattering experiments

The QENS experiments were performed using the disk chopper time-of-flight spectrometer IN5 at the Institut Laue–Langevin (ILL), Grenoble, France. The spectra were measured using incident neutrons of a wavelength of 5 Å at energy resolution of 110 μeV and at 300 K. The measured range of the momentum transfer Q (= $4\pi \sin\theta/\lambda$, where 2θ denotes scattering angle and λ the wavelength of the incident neutrons) were between 0.45 $Å^{-1}$ and 2.1 $Å^{-1}$. The measured time-of-flight spectra were normalized using the vanadium standard, corrected for transmission, subtracted the contribution from the sample can, and transformed into the energy spectra. The obtained spectra were scaled by the amount of actin in the sample can. Data reduction was done with the ILL software package LAMP [7].

3. Results and discussion

The measured QENS spectra can be approximated by the following equation [8]:

$$S(Q,\omega) = DW(Q) \times \exp(-\hbar\omega/2k_BT) \times [(A_0(Q)\delta(\omega) + \sum_{i=1}^{N} A_i(Q)L_i(\omega,\Gamma_i)) \otimes R(Q,\omega)] + B(Q). \quad (1)$$

Here, $\hbar\omega$ is the energy transfer, $DW(Q)$ denotes the Debye–Waller factor which represents the vibrational motions, $\exp(-\hbar\omega/2k_BT)$ is the detailed balance factor, and $A_0(Q)\delta(\omega)$ is the elastic component with $A_0(Q)$ being the fractional intensity and $\delta(\omega)$ being the delta-function. The sum of the components containing the Lorentzians, $L_i(\omega,\Gamma_i) = (1/\pi) \times (\Gamma_i/(\Gamma_i^2 + \omega^2))$, where Γ_i is the half-width at half-maximum), represents the diffusive motions, with $A_i(Q)$ being the fractional intensity of the i-th Lorentzian and N being the number of Lorentzians. $R(Q,\omega)$ is the resolution function, $\otimes$ denotes the convolution operation, and $B(Q)$ is the background. The signal measured on the actin powder hydrated with D_2O arises mainly from the internal dynamics of the actin molecules, as the intensity related to D_2O dynamics can be neglected compared to the protein signal. It can therefore be approximated by the equation containing two Lorentzians [6]. The spectra of the F-actin powders hydrated with D_2O at h = 0.4 and 1.0 at Q = 1.78 $Å^{-1}$, are shown, as examples, in Fig. 1(A) and (C), respectively. The results of the fits with the equation containing two Lorentzians are also shown.

The spectra of the actin powders hydrated with H_2O contain, in addition to the components arising from the internal dynamics of the protein, the components arising from the dynamics of the hydration water. The theoretical dynamical structure factor of the hydration water can be approximated by the following equation [8]:

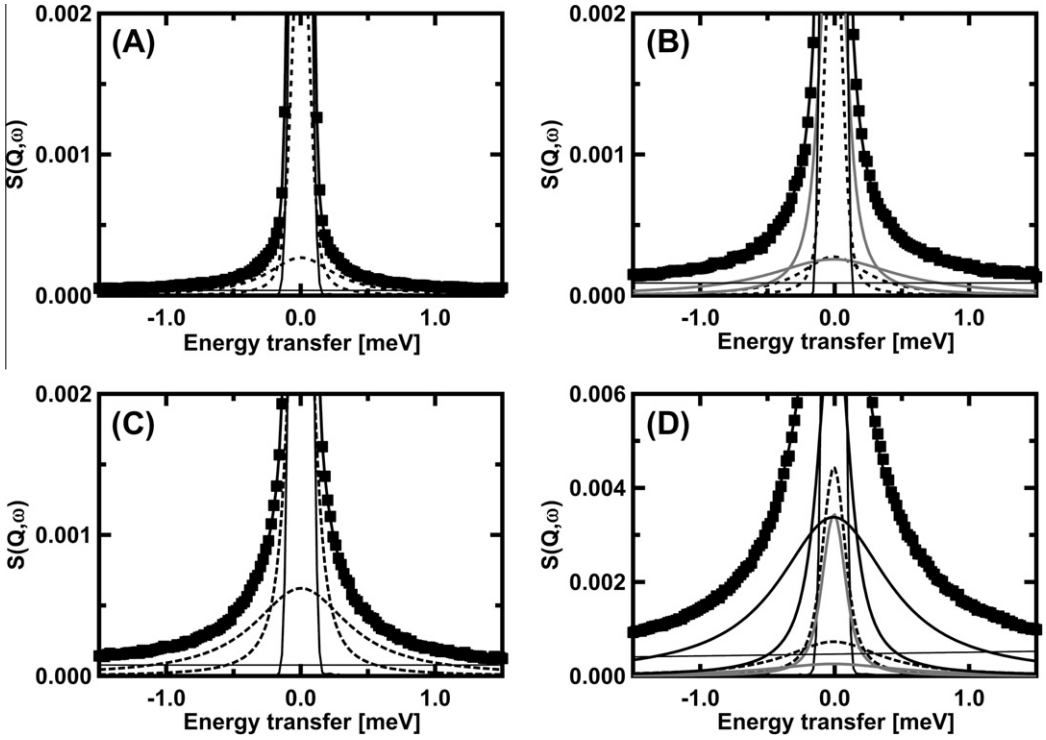

Fig. 1. Examples of the QENS spectra, S(Q,ω), of the hydrated powders of actin. The spectra of (A) the D_2O-hydrated powders of F-actin at h = 0.4, (B) the H_2O-hydrated powders of F-actin at h = 0.4, (C) the D_2O-hydrated powders of F-actin at h = 1.0, and (D) the H_2O-hydrated powders of F-actin at h = 1.0, measured at the temperature T = 300 K and at Q = 1.78 $Å^{-1}$, are shown. Solid squares denote the data points, and the error bars are within the symbols if not shown. The results of the fits with Eq. (1) are also shown. Solid lines around the energy transfer = 0.0 meV denote the resolutions functions, thin horizontal solid lines denote the background, and thick solid lines near the data points denote the fitting curves. In (A) and (C), the spectra were fit with the equation containing two Lorentzians. Dashed lines denote these Lorentzian components of the fits. In (B), the spectra were fit with two additional contributions with respect to (A), accounting for the first layer of the hydration water. Grey solid lines denote these two Lorentzians. In (D), the spectra were fit with the equation containing six Lorentzians. Two contributions arising from the internal dynamics of actin are taken from (C) (dashed lines), the two other contributions arising from the first layer hydration water are taken from (B) (grey lines), and solid lines denote the remaining two Lorentzians, arising from the dynamics of the water molecules outside of the first layer.

$$S_{\mathrm{hydration}}(Q,\omega) = A_1(Q) \times (\Gamma_T(Q)/(\omega^2 + \Gamma_T(Q)^2)) + A_2(Q) \times ((\Gamma_T(Q) + \Gamma_R(Q))/(\omega^2 + (\Gamma_T(Q) + \Gamma_R(Q))^2)), \quad (2)$$

where the first Lorentzian arises from the translational diffusion and the second combines the translational and rotational diffusion with the half-widths at half-maximum (HWHM) being $\Gamma_T(Q)$ and $\Gamma_R(Q)$, respectively. $A_1(Q)$ and $A_2(Q)$ denote the fractional intensity of the first and the second components, respectively. The spectra of the actin-powders hydrated with H_2O at $h = 0.4$, which contain only the first layer of the hydration water, can thus be approximated by Eq. (1) containing four Lorentzians, two of which represent the contributions from the internal dynamics of actin and the other two of which represent the contributions from the dynamics of the hydration water. Fig. 1(B) shows, as an example, the spectrum of the F-actin powder hydrated with H_2O at $h = 0.4$ at $Q = 1.78$ Å^{-1}, with the results of the fits. In these fits, the values of the parameters (the fractional intensity and the HWHM) of the two Lorentzians corresponding to the internal dynamics were taken from the fits to the spectra of the F-actin powder hydrated with D_2O at $h = 0.4$, shown in Fig. 1(A). By using these values, the parameters of the two Lorentzians representing the dynamics of the hydration water were obtained. The dynamics of the hydration water in the first layer can thus be characterized.

The hydrated actin-powders at $h = 1.0$ contain more water than the first layer of the hydration water. If the dynamics of these extra water molecules is distinct from those of the water molecules in the first hydration layer, the spectra of the actin powders hydrated with H_2O at $h = 1.0$ should be fit with Eq. (1) containing the components corresponding to the internal dynamics of the protein, the components arising from the hydration water in the first layer, and two Lorentzians accounting for the dynamics of the extra water molecules. Fig. 1(D) shows an example of the spectra of the F-actin powder hydrated with H_2O at $h = 1.0$, with the results of the fits. In these fits, the parameter values of the two Lorentzians corresponding to the internal dynamics were taken from the fits to the spectra of F-actin in D_2O at $h = 1.0$, shown in Fig. 1(C), and those corresponding to the dynamics of the hydration water in the first layer were from the fits to the spectra of F-actin in H_2O at $h = 0.4$, described above. The parameters of the Lorentzians corresponding to the dynamics of the water molecules other than the hydration water in the first layer can thus be determined. The residuals of these fits with the equation containing the contributions from two distinct water populations were systematically better than those of the fits with a model in which all water molecules behave homogeneously (data not shown). This implies that the dynamics of the water molecules in the samples at $h = 1.0$ have at least one distinct component from that of the hydration water in the first layer, that we assign to the water molecules outside of the first hydration layer.

Similar analysis was applied to the spectra of the G-actin powders. The spectra were fit equally well with these equations (data not shown). This combined analysis of the spectra of the actin powders hydrated with H_2O and D_2O at the different hydration ratios thus makes it possible to extract the information on the dynamics of the hydration water in the first layer as well as those of water outside of the first layer.

Fig. 2 shows the plots of the Γ_T and Γ_R values as a function of Q^2. While the Γ_R values in Fig. 2(B) are rather independent of the Q-values, as expected for the rotational diffusion, the Γ_T values in Fig. 2(A) exhibit dependency on Q. This dependency is distinct between the hydration water in the first layer and those outside of the first layer, as well as in F-actin and G-actin. The Q-dependences of the Γ_T values provide information on the translational

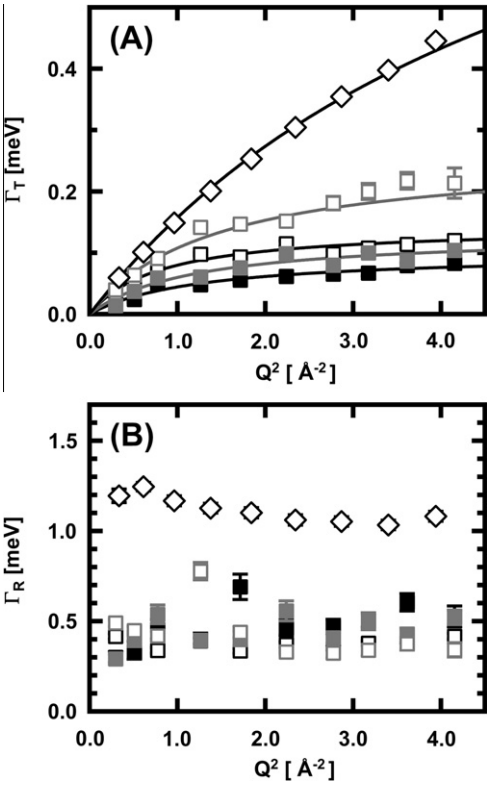

Fig. 2. The Q^2-dependency of half-widths at half-maximum, (A) Γ_T, of the translational diffusion process and, (B) Γ_R, of the rotational diffusion process. Filled squares and open squares in black denote the processes of the hydration water in the first layer and the water outside of the first layer around F-actin, respectively. Filled squares and open squares in grey denote the processes of the hydration water in the first layer and the water outside of the first layer around G-actin, respectively. Open diamond denote the process of bulk water. The parameter values of bulk water were obtained from the fits of Eq. (1) containing two Lorentzians to the spectra of the buffer for F-actin (2 mM Tris–HCl (pH 8.0), 0.2 mM ATP, 0.1 mM $CaCl_2$, 50 mM KCl, and 2 mM $MgCl_2$) in H_2O (the spectra not shown). Since the contributions of salts, contained in this sample, to the QENS spectra are negligible, this sample can be regarded as representing bulk water. The error bars are within the symbols if not shown. Solid lines in (A) represent the results of the fits according to the jump-diffusion model (see text).

diffusion processes. The Γ_T values were best fit with the jump-diffusion model described by

$$\Gamma_T(Q) = D_T Q^2/(1 + D_T Q^2 \tau_0), \quad (3)$$

where D_T denotes the translational diffusion coefficient and τ_0 denotes the residence time between jumps [8]. Solid lines in Fig. 2(A) show the fits with this equation. Also shown in Fig. 2 are the Γ_T and Γ_R values of bulk water measured in similar conditions. Differences in behavior of the Γ_T values and the Γ_R values are observed between hydration water and bulk water.

Table 1 summarizes the parameters obtained by the analysis of the Γ_T values according to the jump-diffusion model. A range of the D_T values of hydration water has been reported, depending on the molecules: for example, 2.6×10^{-5} cm^2/s for fully hydrated C-phycocyanin at 293 K [9], 1×10^{-6} cm^2/s for fully hydrated myoglobin [10], 1.9×10^{-5} cm^2/s for hydrated hen egg-white lysozyme [11], 0.39×10^{-5} cm^2/s for hen egg-white lysozyme crystals [12], 1.4–4.2 $\times 10^{-5}$ cm^2/s for DNA hydrated with H_2O at various degrees [13], and 2.2×10^{-5} cm^2/s for fully hydrated DNA [14]. The values obtained here for the hydration water in the first layer (1.2×10^{-5} cm^2/s for F-actin and 1.7×10^{-5} cm^2/s for G-actin) are within this range. These values are smaller than that of bulk water (2.8×10^{-5} cm^2/s), which agrees with the reported values

Table 1
Summary of the parameters on the translational diffusion of the hydration water.

	The first layer		Outside of the first layer		Bulk water
	F-actin	G-actin	F-actin	G-actin	
D_T ($\times 10^{-5}$ cm^2/s)	1.2 (0.2)	1.7 (0.2)	2.7 (0.2)	2.8 (0.1)	2.8 (0.02)
τ_0 (ps)	6.6 (0.6)	5.0 (0.4)	4.6 (0.2)	2.5 (0.1)	0.62 (0.02)

Values in parenthesis are standard deviations.

[15], indicating reduced mobility of hydration water compared to bulk water. This difference is consistent with the NMR measurements of the hydration water of bovine pancreatic trypsin inhibitor, showing that the translational diffusion is reduced by a factor of two [16]. Furthermore, the value of F-actin is smaller than that of G-actin, indicating that the hydration water around F-actin is less mobile than that around G-actin. The residence times, τ_0, of the hydration water in the first layer around F-actin and G-actin were estimated to be 6.6 ps and 5.0 ps, respectively. These values are comparable to those found in the proteins such as 4.1 ps for C-phycocyanin [9], 5 ps for myoglobin [10], and 11 ps for hen egg white lysozyme crystals [12]. The values for F-actin and G-actin are, however, significantly larger than that of bulk water, 0.62 ps, which is similar to the values in Refs. [17,18] but smaller than that in Ref. [15]. This also implies reduced mobility of the hydration water compared to bulk water. The larger value of the residence time for F-actin than for G-actin indicates again less mobility of the hydration water around F-actin than G-actin.

The results obtained here are in concert with those of the internal dynamics of F-actin and G-actin, showing that G-actin fluctuates more rapidly than F-actin [5,6]. This is in agreement with a coupling of the dynamics of the hydration water and the internal dynamics of the protein, as observed in many protein systems [19]. The sample conditions were, however, not exactly the same in G-actin and F-actin. The main difference was in the composition of salts. The F-actin solutions, from which the hydrated powder samples were prepared, contained 60 mM KCl while the G-actin solutions did not contain such salts. During the preparation of the hydrated powders from these solutions, KCl was concentrated to be close to 3 M in the F-actin samples while the G-actin samples did not contain such high concentrations of salts. Since, however, the translational diffusion coefficients of water in high concentrations of KCl are similar to that of pure water, up to 3.2 M [20], KCl is taken not to affect the behavior of the water molecules. We therefore conclude that dynamics of the hydration water in the first layer are indeed coupled to the internal dynamics of the proteins.

Analysis of the spectra of the samples at $h = 1.0$ made possible to characterize the water dynamics outside of the first hydration layer. To our knowledge, no such analysis has been reported in proteins though it is well accepted that surface water behaves differently from bulk water. The parameter values of these water molecules are shown in the columns "Outside of the first layer" in Table 1. The translational diffusion coefficients of both F-actin and G-actin appear to be very similar to that of bulk water. The residence times are, however, longer than that of bulk water, and, in particular, the residence time of the water molecules around F-actin is longer than that around G-actin. The water molecules naturally exchange between the first hydration layer and the outside, and the rate of this exchange is affected significantly by the residence times of the water molecules in the first layer. This exchange will then have significant effects on the residence times of the water molecules outside of the first layer because the number of the water molecules in the first hydration layer and the outside are comparable (the hydration ratios of 0.4 and 1.0 g water/g protein in the F-actin and G-actin samples indicate that the numbers of the water molecules in the first hydration layer and on the outside are about 900 and 1400 per one actin molecule, respectively). The difference in the residence times between the water molecules outside of the first layer and bulk water arises from this effect. This effect could also account for the differences in residence time of the water molecules outside of the first hydration layer between F-actin and G-actin because the residence time of the first hydration layer is different between F-actin and G-actin.

Interactions between the water molecules in the first hydration layer and those on the outside appear to have similar effects on the rotational diffusion. As shown in Fig. 2(B), the Γ_R values corresponding to the rotational diffusion are similar between the water molecules in the first hydration layer and those on the outside. The rotational correlation time τ_R (=$1/\Gamma_R$) was calculated from the average value of Γ_R of all these data points and equals 3.5 ± 0.2 ps. On the other hand, the value of bulk water was calculated to be 1.5 ± 0.1 ps, which is similar to those reported in Refs. [18,21] but, again, smaller than that in Ref. [15]. This difference in τ_R, corresponding to the reduction of rotational motions, is consistent with the results of the NMR study [16]. Furthermore, it is likely that hydrogen bonds inevitably occurring between the water molecules in the first hydration layer and those on the outside slow down the rotational motions of the water molecules outside of the first hydration layer. It thus appears that the water molecules outside of the first hydration layer behave bulk-like but are still under the strong influence of the behavior of the water molecules in the first hydration layer.

This behavior of the water molecules is consistent with the results of the recent studies on the behavior of intracellular water [18,21,22]. Because of the crowded environment inside cells where more than 300 mg/ml of macromolecules are present [23], only several layers of the water molecules reside between the macromolecules. Most of the cytoplasmic water is, however, shown to behave like bulk water. Although the influence of the first hydration layer on the dynamics of our "outside of the first layer" molecules, which correspond roughly to the second hydration layer, exists, this influence will be less on the subsequent layers because the interactions between the first layer and the subsequent layers are more indirect. One thus expects that "several layers" show bulk-like dynamics. The results obtained here thus provide support for the bulk-like behavior of the cytoplasmic water.

In summary, combined analysis of the QENS spectra of the hydrated powders of F-actin and G-actin in H_2O and D_2O at different hydration ratios made possible to characterize the dynamics of the hydration water in the first layer as well as those of the water molecules outside of the first layer of F-actin and G-actin. It was shown that the dynamics of the hydration water in the first layer around the actin molecules is coupled to the internal dynamics of the actin molecules, and that the water molecules outside of the first hydration layer show behavior like bulk water with longer residence times. The method described here should have wide applicability to various systems.

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