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Difference in the hydration water mobility around F-actin and myosin subfragment-1 studied by quasielastic neutron scattering

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ABSTRACT

Hydration water is essential for a protein to perform its biological function properly. In this study, the dynamics of hydration water around F-actin and myosin subfragment-1 (S1), which are the partner proteins playing a major role in various cellular functions related to cell motility including muscle contraction, was characterized by incoherent quasielastic neutron scattering (QENS). The QENS measurements on the D_2O- and H_2O-solution samples of F-actin and S1 provided the spectra of hydration water, from which the translational diffusion coefficient (D_T), the residence time (τ_T), and the rotational correlation time (τ_R) were evaluated. The D_T value of the hydration water of S1 was found to be much smaller than that of the hydration water of F-actin while the τ_T values were similar between S1 and F-actin. On the other hand, the τ_R values of the hydration water of S1 was found to be larger than that of the hydration water of F-actin. It was also found that the D_T and τ_R values of the hydration water of F-actin are similar to those of bulk water. These results suggest a significant difference in mobility of the hydration water between S1 and F-actin: S1 has the typical hydration water, the mobility of which is reduced compared with that of bulk water, while F-actin has the unique hydration water, the mobility of which is close to that of bulk water rather than the typical hydration water around proteins.

1. Introduction

The actomyosin interaction is a fundamental biological process in a variety of cellular functions related to cell motility including cell locomotion and muscle contraction. Two partner proteins, actin and myosin, are responsible for this interaction, where myosin molecules cyclically interact with F-actin (the polymerized form of actin) utilizing the energy released by hydrolysis of adenosine triphosphate. Many studies suggest that flexibility of F-actin and myosin molecules plays an important role in the actomyosin interaction [1–3]. Our recent study using incoherent quasielastic neutron scattering (QENS) [4] showed that the dynamics of F-actin is different from that of myosin subfragment-1 (S1), which is a proteolytic fragment of the myosin molecule containing the actin-binding site and the catalytic site, such that the atoms of F-actin fluctuate more rapidly than those of S1. F-actin appears to utilize this enhanced mobility to interact with various actin-binding proteins. As proteins reside in an aqueous environment, such fluctuations of proteins occur under the influence of the dynamics of surrounding water molecules. Conversely, the water molecules near the protein surfaces have distinct dynamical properties from bulk water because of the interaction with the proteins. This dynamics of hydration water as well as the protein dynamics plays an active role for proper functions of proteins [5]. Full understanding of the mechanisms of the protein functions thus requires elucidating how the protein dynamics is related to the dynamics of hydration water. For the ultimate purpose of understanding the mechanism of the actomyosin interaction, the relationship between the protein dynamics and the hydration water dynamics in F-actin and S1 should therefore be elucidated. For this purpose, the dynamical properties of hydration water around F-actin and S1 are characterized in the present study.

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The dynamical properties of water molecules are to be characterized in terms of the translational and rotational motions. Studies on the rotational mobility of hydration water around F-actin and S1 using microwave dielectric spectroscopy [6,7] showed that the rotational mobility is higher for the water molecules around F-actin than for those around S1. On the other hand, whether the translational mobility of hydration water around these proteins is different has not been elucidated yet. The translational motions are particularly important because the translational motions of hydration water promote the large-amplitude motions of proteins required for their functions [8]. It is thus essential to directly compare the translational motions as well as the rotational motions of hydration water around S1 and F-actin.

Among various techniques to investigate the dynamics of hydration water, QENS provides a powerful tool to probe directly the motions of water molecules at ps – ns timescales. QENS has been widely used to study the dynamics of hydration water in hydrated protein powders [8–11]. The incoherent neutron scattering cross-section of hydrogen atoms is much larger than that of any other atoms found in biological macromolecules and the isotope deuterium. The signals from water molecules thus dominate in the QENS spectra of solution samples in H_2O-solvent, while those from hydrogen atoms in protein molecules dominate in the spectra of solution samples in D_2O-solvent. Information on the dynamics of the hydration water molecules can be extracted by combined analysis of the QENS spectra of the samples in the H_2O- and D_2O-solvents. In the present study, we compare the dynamics of hydration water around S1 and F-actin in solution using QENS. It was found that both the translational and the rotational mobility of hydration water are higher for F-actin than for S1.

2. Materials and methods

2.1. Sample preparation

S1 and F-actin were purified as described previously [4]. The H_2O-solution samples of these proteins were prepared in the buffer containing 5 mM Tris–HCl (pH 8.0), 0.2 mM ATP, 0.1 mM $CaCl_2$, 1 mM $MgCl_2$, 1 mM NaN_3, and 0.5 mM dithiothreitol (for F-actin), or the buffer containing 20 mM Tris–HCl (pH 8.0), 150 mM KCl, 1 mM NaN_3, and 0.5 mM dithiothreitol (for S1), in H_2O. The concentrations of F-actin and S1 were 151 mg/ml and 75 mg/ml, respectively, determined spectrophotometrically by using the extinction coefficients $E_{280}^{1\%}$ of 11.1 (F-actin) and 7.5 (S1). Each solution sample was put in an aluminum flat cell of 0.2 mm thickness and sealed with indium wire for the QENS experiments. The D_2O-solution samples of F-actin and S1 were prepared in the D_2O-buffer, which contains the same components to those of the H_2O-buffer, except that DCl was used for pD adjustment. The concentrations of F-actin and S1 in the D_2O-buffers were 150 mg/ml and 80 mg/ml, respectively. Note that analysis of the QENS spectra of these D_2O-solution samples was already reported in the previous paper [4].

2.2. Quasielastic neutron scattering experiments

The QENS measurements were carried out using the cold-neutron disk-chopper spectrometer AMATERAS in J-PARC/MLF (Ibaraki, Japan) [12]. Simultaneous measurements of the spectra at the energy resolutions of 90.5, 26.6, and 11.5 μeV (full width at half maximum) were carried out. These energy resolutions correspond to the accessible motions faster than 7, 25, and 57 ps, respectively. The energy resolution thus serves as a motion filter (an instrumental time window) such that the fast motions outside the instrumental time window contribute to a flat background, while the slow motions outside this window are hidden within the instrumental resolution function. Note that the analysis of the QENS spectra at 90.5 and 26.6 μeV energy resolutions are described here. It is because the spectra at 11.5 μeV had poor statistics and the smaller Q-range than those at other energy resolutions (Q is the momentum transfer defined as $4\pi\sin\theta/\lambda$, where 2θ denotes the scattering angle and λ denotes the incident wavelength), and thus were difficult to obtain reliable results by the analysis. A vanadium sample was measured for intensity corrections and for determination of the instrumental resolution functions. The measurement of an empty cell was also carried out, the QENS spectra of which was subtracted from those of all the samples measured. Fitting of the measured QENS spectra was done in the range of $-2.0\ \text{meV} \le \Delta E \le 2.0\ \text{meV}$ using IGOR Pro software (WaveMetrics, Lake Oswego, OR, USA).

2.3. Analysis of the QENS spectra

The QENS spectra of the H_2O-solution samples are dominated by water scattering (bulk and hydration water). To extract the spectra of water, the small but non-negligible contribution from the protein scattering must be subtracted from these spectra. The fractions of the contributions from the proteins and water in these spectra are thus required to be estimated. These fractions can be estimated from the incoherent scattering cross-section σ_{inc}. The values of σ_{inc} per molecule of S1 and water are, for example, calculated from their chemical compositions ($C_{4836}H_{7550}N_{1284}O_{1448}S_{41}$ and H_2O, respectively) to be $603{,}879 \times 10^{-24}\ \text{cm}^2$ and $159.8 \times 10^{-24}\ \text{cm}^2$, respectively. The molar concentration of S1 is calculated from the weight concentration (75 mg/ml) and the molecular weight (108,162) to be $6.93 \times 10^{-7}\ \text{mol/cm}^3$, and that of water is calculated to be $5.08 \times 10^{-2}\ \text{mol/cm}^3$, assuming the partial specific volume of S1 as $0.73\ \text{cm}^3\text{/g}$. Thus, the values of σ_{inc} of S1 and water per unit volume of the sample are $0.25\ \text{cm}^{-1}$ and $5.05\ \text{cm}^{-1}$, respectively, and thereby the fractions of their contributions being 0.05 and 0.95, respectively. Similar calculations provide the fractions of the contributions of F-actin ($C_{1854}H_{2907}N_{493}O_{565}S_{21}$ for a monomer) and water as 0.10 and 0.90, respectively. The fractions of the contributions of the proteins and (heavy) water in the D_2O-solution samples are similarly evaluated, with taking account of the H-D exchange. Assuming that all the exchangeable H atoms are replaced with the D atoms, the chemical compositions of S1 and the actin molecule in D_2O are $C_{4836}H_{5868}D_{1682}N_{1284}O_{1448}S_{41}$ and $C_{1854}H_{2277}D_{630}N_{493}O_{565}S_{21}$, respectively. The fractions of the contributions of the proteins and D_2O were then calculated to be 0.62 and 0.38 for S1, and 0.76 and 0.24 for F-actin, respectively. Using these values as the scaling factors, the QENS spectra of the proteins can be obtained by subtracting the spectra of the D_2O-buffer from those of the D_2O-solution samples [4], and then the QENS spectra of water can be obtained by subtracting these protein spectra from the spectra of the H_2O-solution samples.

It should, however, be noted that the degree of the H-D exchange may not be 100%. It has been suggested that a more reasonable assumption is that about 80% of the exchangeable H atoms are replaced [13]. If, as an extreme case, it is assumed that only 50% of the exchangeable H atoms are replaced with D atoms, the numbers of the D atoms are 841 for S1 and 315 for the actin molecule. In this case, the fractions of the contributions of the proteins and D_2O in the QENS spectra are 0.65 and 0.35 for S1, and 0.79 and 0.21 for F-actin, respectively. From comparison of these values with those assuming the 100% exchanges, possible errors in these fractions are estimated to be at most 5%. Since the fractions of the contributions of the proteins in the H_2O-solution samples are 0.05 and 0.1 for S1 and F-actin, respectively, the errors due to