Difference in hydration structures between F-actin and myosin subfragment-1 detected by small-angle X-ray and neutron scattering

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Received June 5, 2013; accepted June 13, 2013

Hydration structures around F-actin and myosin subfragment-1 (S1), which play central roles as counterparts in muscle contraction, were investigated by small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS). The radius of gyration of chymotryptic S1 was evaluated to be 41.3±1.1 Å for SAXS, 40.1±3.0 Å for SANS in H₂O, and 37.8±0.8 Å for SANS in D₂O, respectively. The values of the cross-sectional radius of gyration of F-actin were 25.4±0.03 Å for SAXS, 23.4±2.4 Å for SANS in H₂O, and 22.6±0.6 Å for SANS in D₂O, respectively. These differences arise from different contributions of the hydration shell to the scattering curves. Analysis by model calculations showed that the hydration shell of S1 has the average density 10–15% higher than bulk water, being the typical hydration shell. On the other hand, the hydration shell of F-actin has the average density more than 19% higher than bulk water, indicating that F-actin has a denser, unusual hydration structure. The results indicate a difference in the hydration structures around F-actin and S1. The unusual hydration structure around F-actin may have the structural property of so-called “hyper-mobile water” around F-actin.

Key words: hydration shell, SAXS, SANS

Proteins in physiological conditions fluctuate constantly under the influence of thermal fluctuations of surrounding solvent molecules, the most abundant of which is water molecules. Proteins achieve their functions by changing their conformations utilizing this thermal energy of the solvent. Elucidation of interactions between proteins and water is thus indispensable for the ultimate understanding of protein functions. A first step toward this goal is to characterize the structural and dynamic properties of hydration water around proteins as well as proteins themselves. In this context, we have investigated the dynamic properties of the protein actin¹–³.

Actin forms a helical polymer (F-actin), and plays crucial roles in a variety of functions related to cell motility. This multi-function of F-actin arises from flexibility of F-actin that enables it to interact with various actin-binding proteins. For understanding the origin of this flexibility and thereby the mechanism of the multi-functions, characterization of the structural and dynamic properties of F-actin including hydration water is required. In particular, investigation of hydration water around F-actin is important because it has been suggested that water plays an important role in chemomechanical energy transduction during muscle contraction⁴,⁵. Furthermore, the water molecules around F-actin has been suggested to have unusual properties (higher rotational mobility than bulk water), compared to usual hydration water that have lower rotational mobility than bulk water⁶.

We employed small-angle X-ray and neutron scattering techniques to investigate the structural properties of hydration water around F-actin and myosin subfragment-1 (S1),
which is a proteolytic fragment of the myosin molecule containing a motor domain that interacts with actin in muscle contraction. Small-angle scattering arises from the difference in scattering density (the contrast) between the particles of interest and solvent. Signs and amplitudes of the contrasts of proteins and hydration water are different between small-angle X-ray scattering (SAXS), small-angle neutron scattering (SANS) in H$_2$O, and SANS in D$_2$O: in SAXS, the contrast of proteins in solution is positive and that of hydration water, assuming higher density than bulk water, is also positive; in SANS in H$_2$O, the contrast of proteins is positive, but that of hydration water is negative with small amplitudes; and in SANS in D$_2$O, the contrast of proteins is negative while that of hydration water is positive. Utilizing these differences, combined analysis of the measurements by SAXS, SANS in H$_2$O, and SANS in D$_2$O provides information on the hydration structure of proteins.

The SAXS and SANS measurements were performed on F-actin in H$_2$O and D$_2$O to investigate the hydration structure. Similar measurements were also done on S1. Comparison of the results of F-actin and S1 indicates the denser hydration shell around F-actin than that around S1.

1. Materials and methods

1.1. Sample preparation

Actin was extracted from chicken breast muscle (pectoralis) and purified as described. Further purification was done by gel filtration using a column (HiLoad 26/600 Superdex 200 pg; GE healthcare) in solution containing 5 mM Tris-HCl (pH 8.0), 0.2 mM ATP, 0.1 mM CaCl$_2$, 1 mM NaN$_3$, and 0.5 mM dithiothreitol (G-buffer), in which the actin molecules are monomeric (G-actin). To prepare the samples in D$_2$O, additional dialysis against the G-buffer in D$_2$O was performed. The F-actin samples were prepared by polymericization of G-actin by adding MgCl$_2$ to 1 mM$^2$ just before the scattering measurements. The protein concentration was determined spectrophotometrically by using the extinction coefficients, $E_{280}$ of 6.3 for G-actin and $E_{280}$ of 11.1 for F-actin.

S1 was prepared by chymotryptic digestion of myosin from rabbit skeletal muscle as described. Purification of S1 obtained was done by gel filtration using a column (HiLoad 26/600 Superdex 200 pg; GE healthcare) in solution containing 20 mM Tris-HCl (pH 8.0), 150 mM KCl, 1 mM NaN$_3$, and 0.5 mM dithiothreitol. This chymotrypsin-treated S1, the molecular weight of which is 110 kDa, consists of the heavy chain (lacking the regulatory light chain-binding portion) and the essential light chain. The samples in D$_2$O were prepared by additional dialysis against the D$_2$O solution containing the same salt compositions as above. The concentration of S1 was determined spectrophotometrically by using $E_{280}$ of 7.5.

1.2. Small-angle scattering experiments

The SAXS measurements were carried out at the beamline BL45XU-SAXS at SPring-8, Hyogo, Japan, using incident X-rays with the wavelength ($\lambda$) of 1.0 Å. The measurements on a concentration series of the samples (F-actin in H$_2$O, F-actin in D$_2$O, S1 in H$_2$O and S1 in D$_2$O) between 1 mg/ml and 5 mg/ml were performed at the sample-to-detector distance of 2.5 m at 293 K. Two-dimensional SAXS patterns were measured with a PILATUS 300K-W detector (DECTRIS), then circularly averaged to obtain one-dimensional scattering curves using the program FIT2D. Background subtraction was done using the program Primus to obtain net scattering curves. Each scattering curve was obtained by accumulating three sets of scattering data from 500-ms exposure, during which no radiation-damage effects were observed.

The SANS measurements were carried out on the small-angle diffractometer D22 at the Institut Laue-Langevin, Grenoble, France, using neutrons with $\lambda = 6$ Å ($\Delta \lambda / \lambda = 10\%$). The measurements were done at the sample-to-detector distance of 5.6 m at 293 K. Exposure time was 10–20 min for each sample. Data reduction to obtain one-dimensional net scattering curves was carried out using the program GRASP.

1.3. Guinier analysis of the scattering curves

Guinier analysis of the scattering curves was done to evaluate the radius of gyration ($R_g$) for S1 or the cross-sectional radius of gyration ($R_c$) for F-actin. The scattering curve, $I(Q)$, where $Q = 4\pi\sin(\theta) / \lambda$, where $2\theta$ is the scattering angle) denotes the momentum transfer, can be approximated at the Guinier region ($QR_g < 1.3$) of the scattering curves as,

$$I(Q) = I(0) \exp(-R_g^2 Q^2/3),$$

where $I(0)$ denotes the intensity at $Q = 0$. A linear fit to the Guinier plot (the plot of $\ln[I(Q)]$ versus $Q^2$) provides the values of $R_g$ from the slope and $I(0)$ from the intercept at $Q = 0$.

For rod-like particles such as F-actin, the scattering curves have an approximate form,

$$I(Q) = I_c(Q) Q,$$

where $I_c(Q)$ denotes the scattering curve of the cross-section of the particle. Since the Guinier region of $I_c(Q)$ can be approximated as,

$$I_c(Q) = I_c(0) \exp(-R_c^2 Q^2/2),$$

the $R_c$ and $I_c(0)$ values can be evaluated from a linear fit to the cross-sectional Guinier plot (the plot of $\ln[I_c(Q)]$ versus $Q^2$).

1.4. Model calculations

Further analysis was done by model calculations based on atomic models obtained by X-ray crystallography, X-ray
fiber diffraction (XFD), or three-dimensional reconstruction of electron microscopy (EM) images. For S1, the crystal structure of S1 prepared by papain digestion of myosin (PDB ID: 2MYS13) was used. The papain-treated S1 consists of the heavy chain, the essential light chain, and the regulatory light chain. The heavy chain forms the catalytic domain and the lever arm, which is a long stretch of α-helix of the heavy chain extending from the catalytic domain. The essential light chain and the regulatory light chain are wrapped around this α-helix of the heavy chain. The chymotrypsin-treated S1 used here, on the other hand, does not contain the regulatory light chain and its corresponding region in the heavy chain. The atomic model of S1 was thus modified so that the C-terminal residues from Cys12 of the heavy chain and the regulatory light chain were removed. In addition, the missing side chains in the essential light chain in the crystal structure were added to the Cα coordinates manually. The extra methyl groups were also removed from the methylated lysine residues in the crystal structure. The missing residues in the crystal structure were complemented using a template-based loop structure prediction server ArchPRED16.

The scattering curve calculated from the crystal structure of S1 was found not to fit well to the experimental curve regardless of the hydration shell (data not shown). A SAXS study on the myosin-family proteins, myosin V and VI, indeed showed that the structures of S1 in solution is different from those in crystals17. Global search of the structure of S1 thus needed to be performed. Since major structural changes have been shown to occur in the orientation of the lever arm relative to the catalytic domain17,18, the global search of the lever arm orientation was performed. Each of three Euler angles describing the orientation of the lever arm (the residues 707–814 in the heavy chain and the essential light chain) around the residue Cys70719 was changed with a step of 30°, and for each orientation, the scattering curves were calculated using the programs CRYSTAL and CRYSON20. CRYSTAL and CRYSON include the contribution of the first hydration shell with a thickness of 3 Å in the calculation of scattering curves, taking the relative contrast to bulk water as a parameter. The global search was thus performed at various relative contrasts of the hydration shell. At each of the contrasts between 0% and 40% with a step of 1%, the global search of the orientation of the lever arm was performed to determine the model which fits simultaneously to the SAXS data, the SANS data in H2O, and the SANS data in D2O by minimizing the score function, P:

\[
P = \chi^2_{\text{SANS}} + \chi^2_{\text{SANSH}} + \chi^2_{\text{SANSD}}
\]

\[
\chi^2 = (1/(N-1)) \sum_{i=1}^{N} \left( (I_{\text{exp}}(Q) - I_{\text{calc}}(Q))/\sigma_{\text{exp}}(Q) \right)^2,
\]

where the subscripts SANS, SANSH, and SANSD denote the SANS data in H2O and the SANS data in D2O, respectively, N is the number of the data points, \(I_{\text{exp}}(Q)\) and \(I_{\text{calc}}(Q)\) are the experimental and calculated scattering intensity at \(Q\), respectively, \(c\) is the scale factor for the calculated curves, defined as \(I_{\text{exp}}(0)/I_{\text{calc}}(0)\), and \(\sigma_{\text{exp}}(Q)\) is the experimental error at \(Q\). The fits were performed to the extrapolated scattering curves to zero protein concentration, obtained from a concentration series of the experimental scattering curves. In this manner, both the relative contrast of the hydration shell and the model of S1 can be determined.

For F-actin, three atomic models of the actin molecule obtained from XFD studies (PDB ID: 2ZWH15 and EM studies of F-actin (PDB ID: 3MFP22 and 3G3723) were used. The F-actin models were constructed as 13-mers (for 2ZWH and 3MFP), or 12-mers (for 3G37) of the actin molecules from these models, assuming the left-handed 13/6 helical symmetry as was done previously24. Since these structures were based on the models obtained from the XFD or EM measurements of the “solution” samples, the structures should be free from the distorting molecular forces such as crystal-packing force. Thus, the structural search of the actin molecule in F-actin was not performed. At each of the relative contrasts of the hydration shell between 0% and 50% with a step of 1%, the scattering curves of the models for SAXS, SANS in H2O, and SANS in D2O were calculated using CRYSTAL and CRYSON. The \(R_c\) values of the models were then obtained from the cross-sectional Guinier plots of these curves. The relative contrast of the hydration shell was determined so that the discrepancy between the experimental and calculated \(R_c\) values was minimized using the score function, \(S\), defined as,

\[
S = \xi_{\text{SANSH}} + \xi_{\text{SANSH}} + \xi_{\text{SANSD}},
\]

\[
\xi = \sqrt{(R_c^{\text{exp}} - R_c^{\text{calc}}(h))/\sigma_{\text{exp}}^2},
\]

where \(R_c^{\text{exp}}\), \(\sigma_{\text{exp}}\), and \(R_c^{\text{calc}}(h)\) denote the experimental \(R_c\), the standard deviation of \(R_c^{\text{exp}}\), and the calculated \(R_c\) at the contrast of the hydration shell, \(h\), respectively.

2. Results and discussion

2.1. Small-angle scattering experiments

Figure 1A and B shows examples of the Guinier plots of S1, and the cross-sectional Guinier plots of F-actin, respectively. Figure 1C and D shows the concentration dependences of the \(R_c\) values of S1 and the \(R_c\) values of F-actin, respectively. Linear extrapolation of these values to zero protein concentration provides the values at infinite dilution, which are devoid of distorting effects of the inter-particle interference. Although the isotopic effects of D2O should not be observed in SAXS, the SAXS data of the samples in D2O as well as in H2O were measured because the inter-particle interference could be different between the particles in H2O and those in D2O. The obtained \(R_c\) and \(R_c\) values were, however, similar in H2O and D2O as shown in Figure 1C and D. Thus it was concluded that the differences between the SAXS data in H2O and in D2O are negligible.
The extrapolated $R_g$ values of S1 and $R_c$ values of F-actin are summarized in Table 1. The values obtained from SANS in D$_2$O were smaller than those from SANS in H$_2$O, which were then smaller than those from SAXS. This relationship is consistent with that which is expected if the hydration shell has higher density than bulk water. The $R_g$ values of S1 obtained here are consistent with the earlier SAXS studies though the $R_g$ value from SANS in D$_2$O is slightly smaller than the reported value of 40.0±1.5 Å in the earlier SANS study. The $R_g$ values of F-actin are also consistent with the earlier SANS study and SAXS studies.

### Table 1

|             | $R_g$ of S1 (Å) | $R_c$ of F-actin (Å) |
|-------------|-----------------|----------------------|
| SAXS        | 41.3 (1.1)      | 25.4 (0.03)          |
| SANS in H$_2$O | 40.1 (3.0)   | 23.4 (2.4)           |
| SANS in D$_2$O | 37.8 (0.8)   | 22.6 (0.6)           |

Values in parenthesis are standard deviations.

2.2. Model calculation for obtaining the hydration structure around S1

To evaluate the relative contrast of the hydration shell around S1 and obtain the structure of S1, global search for the orientation of the lever arm and the contrast of the hydration shell was performed. Figure 2A shows the contrast-dependence of the score function, $P$ of equation (1) in a range between 0% and 20%. (The range above 20% was omitted because of monotonic increase in $P$ with increasing the contrast.) $P$ has a rather flat region in a range between 10% and 15% with the minimum at 14%. Figure 2B shows the comparison of the experimental scattering curves and the scattering curves calculated from the model at the minimum $P$. The good fits to the experimental curves were obtained in the range of 0.015 < $Q$ < 0.15 Å$^{-1}$. The values of the residual $\chi^2$ of equation (4) of this model were 1.24 for SAXS, 1.02 for SANS in H$_2$O, and 1.88 for SANS in D$_2$O. The $R_g$ values of the models were 40.6 Å for SAXS, 39.3 Å for SANS in H$_2$O, and 38.5 Å for SANS in D$_2$O. These $R_g$ values were within a range of one standard deviation of the measured $R_g$ values (see Table 1). For the models at the contrasts out of the range between 10% and 15%, at least...
one of the $\chi^2$ values became larger than 2.0 in addition to worse values of $P_i$, indicating that the simultaneous fits to the scattering curves failed at the contrasts below 10% and above 15%. The acceptable range of the contrast was thus between 10% and 15%, consistent with the earlier result that S1 had usual hydration water\(^{31}\). An ensemble of the models in the acceptable range of the contrast shows different orientations of the lever arm from that of the crystal structure, though such a difference was not observed in the earlier study.\(^{26}\) The origin of this discrepancy is not clear at present. Slight differences in the conditions for chymotrypsin treatment, however, may have caused digestion of a few more residues in the motor domain, which could unstabilize the interaction between the lever arm and the motor domain at their junction and thereby changing the orientation of the lever arm as detected here. Such a local perturbation is unlikely to modify the overall hydration structure significantly.

2.3. Model calculation for obtaining the hydration structure around F-actin

The $R_c$ values of F-actin for SAXS, SANS in H\(_2\)O, and SANS in D\(_2\)O were calculated at each of the contrasts between 0% and 50% with a step of 1%, using the atomic models of F-actin. The contrast-dependence of these $R_c$ values obtained from the calculations is shown in Figure 3A. Although these calculations were performed using the 13-mers or 12-mers of the F-actin models, such calculations using the 10-mers, 26-mers and 39-mers of the F-actin models confirmed that the $R_c$ values did not depend on the number of monomers (data not shown). Figure 3B shows the contrast-dependence of the score function, $S$ of equation (5) calculated from these $R_c$ values. The model based on the EM structure of 3MFP (3MFP-EM) reached the minimum value of $S$ at the contrast of 20%, at which the $R_c$ values are 25.4 Å for SAXS, 24.1 Å for SANS in H\(_2\)O, and 22.8 Å for SANS in D\(_2\)O. These values are within a range of one standard deviation of the corresponding experimental $R_c$ values. On the other hand, the model based on the XFD structure of 2ZWH (2ZWH-XFD) reached the minimum value of $S$ at the contrast of 36%, at which the $R_c$ values are 25.4 Å for SAXS, 23.4 Å for SANS in H\(_2\)O, and 21.6 Å for SANS in D\(_2\)O. Another model based on the EM structure of 3G37 (3G37-EM) reached the minimum at the contrast of 40%, at which the $R_c$ values are 25.4 Å for SAXS, 23.2 Å for SANS in H\(_2\)O, and 20.9 Å for SANS in D\(_2\)O. The $R_c$ values of the 2ZWH-XFD and 3G37-EM models are also within a range of one standard deviation of the experimental values except for the values from SANS in D\(_2\)O. Assuming that the acceptable range of the calculated $R_c$ values are within twice the standard deviations of the experimental values, the acceptable range of the contrast was between 19% and 21% for the 3MFP-EM model, and between 34% and 38% for the 2ZWH-XFD model. The fits using the 3G37-EM model did not satisfy this criterion because the $R_c$ value from SANS in D\(_2\)O was out of the range of twice the standard deviation.

The atomic models of F-actin used here should be free from the distorting molecular force. The difference in the optimal contrast of the hydration shell among these models arises from slight differences in their conformations as shown in Figure 4. When F-actin models used in this study are helically projected onto the plane perpendicular to the fiber axis and viewed from the pointed-end, the 3MFP-EM model is shown to be more expanded as a whole than the 2ZWH-XFD model although the centers of mass of these models virtually coincide with each other (Fig. 4A). On the other hand, the 3G37-EM model is the closest to the fiber axis among these three models, and the relative position of the inner and the outer domains within an actin monomer is slightly different from that in other two models (Fig. 4B and...
C). F-actin was demonstrated to have a range of structural conformations, which are distinct in local structures, but overall structures of which are similar to the 2ZWH-XFD model. F-actin in solution is thus likely to have an ensemble of conformations similar to the three models above. Since the most expanded structure is that of the 3MFP-EM model, the relative contrast 19–21% higher than bulk water obtained from the modeling should be the minimum contrast. It is thus concluded that the contrast of the hydration shell around F-actin is at least 19% higher than bulk water and it could be more than 30%. This value is significantly higher than the value for S1.

Svergun et al. first applied the technique employed here to protein solutions and concluded that the hydration shell around the protein has on average the density ~10% higher than that of bulk water. A molecular dynamics simulation study showed that the density of the hydration shell around hen egg white lysozyme is 15% higher than bulk water. Furthermore, a recent analysis of small- and wide-angle X-ray scattering of protein solutions suggested that the contrast of the hydration shell around myoglobin is about 10% higher and solvent analysis of insulin crystal showed the hydration shell with 10% higher density than bulk water. Taking these and other evidences into consideration, the first hydration shell around the proteins is considered to have an average density of at most 10% higher than that of bulk water. The results of the hydration shell around S1 obtained here fit into this general picture. On the other hand, F-actin, having the hydration shell with more than 19% higher density than bulk water, is out of this picture. F-actin thus has an unusual hydration structure.

This hydration structure around F-actin could be interpreted as follows. F-actin has been shown to have clusters of negative charges on the solvent-accessible surface. These negative charges produce the electric field, the strength of which is comparable to that around an iodide ion. An iodide ion has been shown to make the water structure around the ion highly disordered. Furthermore, the number of hydrogen-bonds per water molecule is significantly reduced around the iodide ion compared to bulk water. Such disordered water structure increases the density of the hydration shell. The clusters of the negative charges around F-actin should thus have the similar effects of making the water structure disordered and thereby increasing the density of the hydration shell.

In such a disordered hydration shell, each water molecule may rotate more rapidly than that in bulk water despite of high density because of the disrupted hydrogen-bond network. The hydration shell with high density around F-actin could thus be related to the unusual behavior of the water.

Figure 3 (A) Dependence of the \( R_c \) values of the F-actin models on the contrast of the hydration shell. The calculated \( R_c \) values based on the models of 2ZWH-XFD, 3MFP-EM and 3G37-EM are shown. (B) Dependence of the score function, \( S \), on the contrast of the hydration shell. The values of \( S \) of the models of 2ZWH-XFD, 3MFP-EM and 3G37-EM are shown.
molecules around F-actin, which was suggested by a microwave dielectric spectroscopic study\(^6\) that the water molecules around F-actin have higher rotational mobility than bulk water (the hyper-mobile water, HMW). The results of the earlier studies showing that the HMW was detected around iodide ions\(^6\) but not around the proteins having usual hydration water such as S1, myoglobin, and lysozyme\(^31,40\) may be in concert with the relevance of the unusual density of the hydration shell to the HMW.

The results obtained here indicate that the hydration structures were different between F-actin and myosin S1, which are counterparts in muscle contraction. Changes in the hydration structure would play important roles in protein-protein interactions, as suggested for the acto-S1 interaction.\(^6,31\) Systematic studies on hydration water around various proteins are required to elucidate the roles which hydration water plays in a variety of protein functions.

**Acknowledgements**

We thank Dr. T. Hikima for his help during the experiments at BL45XU, SPring-8 and Drs. L. Porcar, A. Martel, H. Nakagawa, H. Endo, and Prof. G. Zaccai for their help during the experiments at D22, ILL. We also thank Prof. K. Wakabayashi for discussions. This work was supported in part by Grant-in-Aid for Scientific Research on Innovative Areas from the Ministry of Education, Culture, Sports, Science and Technology (to SF).

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