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Biochemical and Biophysical Research Communications 322 (2004) 340–346
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Myosin-induced volume increase of the hyper-mobile water surrounding actin filaments

Makoto Suzuki[a,*], Syed Rashel Kabir[a], Md. Shahjahan Parvez Siddique[a], Umme Salma Nazia[a], Takashi Miyazaki[a], Takao Kodama[b]

[a] *Department of Materials Science and Engineering, Tohoku University, Aoba-yama 02, Sendai 980-8579, Japan*
[b] *Laboratory of Molecular Enzymology, Faculty of Information Science and Systems Engineering, Kyushu Institute of Technology, Ihzuka, Fukuoka 820-8502, Japan*

Received 5 July 2004
Available online 7 August 2004

Abstract

Microwave dielectric spectroscopy can measure the rotational mobility of water molecules that hydrate proteins and the hydration-shell volume. Using this technique, we have recently shown that apart from typical hydrating water molecules with lowered mobility there are other water molecules around the actin filaments (F-actin) which have a much higher mobility than that of bulk water [Biophys. J. 85 (2003) 3154]. We report here that the volume of this water component (hyper-mobile water) markedly increases without significant change of the volume of the ordinary hydration shell when the myosin motor-domain (S1, myosin subfragment-1) binds to F-actin. No hyper-mobile component was found in the hydration shell of S1 itself. The present results strongly suggest that the solvent space around S1 bound to F-actin is diffusionally asymmetric, which supports our model of force generation by actomyosin proposed previously [op. cit.].

Keywords: Hyper-mobile water; Microwave dielectric spectroscopy; Protein hydration; Actomyosin; Linear motor mechanism; Actin filament; Negative hydration; Water structure breaker; Brownian ratchet; Urea hydration

Protein molecules fold and function in aqueous environments, where there exist three broad categories of water molecules, which are in order of mobility, strongly bound, internal water molecules that occupy internal cavities and deep clefts; water molecules that interact with the protein surface; and bulk water [1,2]. Recently, we have shown the presence of another category of water (hyper-mobile water) around F-actin, which has a much higher mobility than that of bulk water but co-exists with ordinary hydrating water of lower mobility [3]. The method we have used is microwave dielectric spectroscopy, which can measure the rotational mobility of water molecules that hydrate proteins and the volume of the hydration shells [4,5]. The rotational mobility is reflected as the dielectric relaxation frequency, f_c which gives the dielectric relaxation time $\tau_{diel}(1/2\pi f_c)$. The f_c values estimated were ~40 GHz for hyper-mobile water and <8 GHz for ordinary hydrating water, which are compared to 17 GHz for bulk water at 20 °C [6]. The volume fraction of the hyper-mobile water is as large as 80% of the molecular volume of G-actin in size, which is larger than that of the ordinary hydration shell (Table 1).

Solutions of urea and potassium-halides, KCl and KI, which are known to exhibit water-structure breaking effects [7] were also studied. The results indicated the presence of fast components with f_c values of 30–40 GHz for urea and 25–30 GHz for potassium-halides. In addition, we have recently found the f_c value of 15–20 GHz for the hyper-mobile component in urea D_2O solution at 5 °C where the f_c for bulk water (D_2O) is

* Corresponding author. Fax: +81 22 217 7303.
E-mail address: msuzuki@material.tohoku.ac.jp (M. Suzuki).

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doi:10.1016/j.bbrc.2004.07.111

Table 1
Dielectric parameters for hydration of actin, S1, and acto-S1

	1st relaxation component				2nd relaxation component			
	f_{c1} (GHz)	δ_1	ϕ_1/c (ml/g)	N_1[b]	f_{c2} (GHz)	δ_2	ϕ_2/c (ml/g)	N_2[b]
F-actin[a]	6.5	45	0.506	1190	40	75	0.601	1410
S1 without ADP ($n = 12$)	7.2 ± 0.4	69 ± 5	0.565 ± 0.011	3770 ± 87[c]	—	—	—	—
S1 with ADP ($n = 4$)	6.6 ± 0.4	69 ± 4	0.562 ± 0.017	3750 ± 98[c]	—	—	—	—
Acto-S1(ADP) ($n = 6$)	5.9 ± 0.2	47 ± 2	0.511 ± 0.020	1726 ± 62	39 ± 2	76 ± 2	0.770 ± 0.038	2600 ± 119
Calculated	—	—	0.507	1710	—	—	—	—

The values are means of n measurements with standard errors.

[a] Data from [3].

[b] The number of water molecules in the hydration shell (N_1 or N_2) was calculated by using a general equation, $N_i = n_w\phi_i/n_p$, where n_p and c are the protein concentrations in (mol/L) and (g/ml), respectively, and n_w is the molar concentration of pure water (=55.6 mol/L).

[c] Calculation was made by using the weight-averaged value of 60,760 ($= (13M_r^{actin} + 2M_r^{S1})/13$) for molecular mass of acto-S1(ADP) complex, where $M_r^{actin} = 42,300$ and $M_r^{S1} = 120,000$.

10.3 GHz (T.M., M.S., and D. Kokubo, unpublished data). Taken together, our dielectric method is capable of revealing the unique hydration properties of actin, and the hyper-mobile water inducing effect may be synonymous with water-structure breaking.

It should be emphasized that no hyper-mobile water has been observed for any other globular proteins examined so far [3,4]. Furthermore, fast hydration dynamics at the protein surface has been recently studied using a multitude of physicochemical methods and molecular dynamics simulations (see [8,9] for recent reviews). Some result of proton-spin relaxation measurement suggested that the rigor cross-bridge formation disrupts the structured water bound to myosin and actin filaments in muscle fiber [10]. To the best of our knowledge, however, no description based on these studies has ever been made of water around the protein surface whose mobility exceeds that of bulk water.

In order to inquire any functional implications of hyper-mobile water, it is of primary importance to study how actin-binding proteins affect such a unique feature of actin hydration, because the actin participates in various processes vital to living cells through interaction with these proteins [11]. This report is concerned with the myosin motor-domain (S1), since its interaction with actin (acto-S1 interaction) is the fundamental basis of muscle contraction and hence has been well characterized by various physicochemical methods [12,13].

Materials and methods

Proteins. Actin was prepared from chicken pectoral and leg muscle [14] and concentrated with Aquacide II (Calbiochem). Myosin prepared from the pectoral muscle [15] was digested by papain to prepare S1 [16], which was purified by DEAE–cellulose chromatography [17] and concentrated by precipitation with ammonium sulfate followed by dissolving in a small amount of buffer. Proteins prepared were subjected to SDS–PAGE analysis, which assured a purity of >98%. The Mg-ATPase activity was measured at pH 7.2, 20 °C by the malachite green method [18]. The S1 activity was 0.05–0.06 s^{-1} and the actin-activated one was 7.0 s^{-1}. Protein concentrations were determined from absorption at 280 nm with $A^{1\%}_{280\,nm} = 7.5\,cm^{-1}$ [17] for S1 and 290 nm with $A^{1\%}_{290\,nm} = 6.3\,cm^{-1}$ [11] for actin. The values of partial specific volume (s_v) for F-actin, acto-S1 filament ([actin]:[S1]=13:2), and S1 were 0.74, 0.73, and 0.73 ml/g, respectively, which was calculated from the density of their solutions measured with an Anton-Paar DMA-58 density meter.

Sample preparation. Acto-S1 solutions for dielectric spectroscopy were prepared by the two-step procedure. First, actin (~16 mg/ml) in 2 mM Hepes (pH 7.0) containing 0.1 mM ATP and 0.1 mM $CaCl_2$ was polymerized by adding KCl and $MgCl_2$ to 50 and 2 mM, respectively. S1 (~18 mg/ml) in 2 mM Hepes (pH 7.0) containing 0.1 mM $CaCl_2$, 50 mM KCl, and 2 mM $MgCl_2$ was then added and mixed. To ensure a thorough mixing of the proteins at such high concentrations, the ATP concentration was raised to 0.2 mM just prior to mixing. The G-actin-unit/S1 molar ratio adopted was 13:2, which roughly corresponds to the state of an actin filament fully overlapping with myosin in muscle. It was difficult to make accurate measurements at higher ratios (the solution became turbid).

Dielectric spectroscopy. All measurements were made in a conically shaped glass cell (total volume, 3.2 ml) connected to a microwave network analyzer at 20.0 ± 0.01 °C (Hewlett Packard 8720C-85070A). The frequency (f)-dependent signals were converted into complex dielectric spectrum consisting of real and imaginary parts, $\varepsilon^* = \varepsilon' - i\varepsilon''$ (Figs. 1A and B), which were analyzed as previously described [3,4]. In short, the spectra of the buffer ($\varepsilon^*_w(f)$) and protein solution ($\varepsilon^*_{ap}(f)$) were measured in pairs 4–12 times at a given protein concentration. The difference was taken for each pair (difference spectrum $\Delta\varepsilon^*(f)$, protein solution vs. solvent buffer). The difference spectra obtained were then averaged and subjected to mathematical smoothing to reduce the noises of various sources, which were then analyzed with a randomly oriented ellipsoidal/double-shelled-solute model [19]. This procedure enables our measurement to resolve 0.01 of $\Delta\varepsilon'$ and 0.02 of $\Delta\varepsilon''$ over the frequency range from 2 to 20 GHz. The axial ratio of the solute was assumed to be 30 for F-actin and 3.0 for myosin S1. The dielectric constant of solute (ε^*_q) as a function of f was approximated by a Debye relaxation function with relaxation frequency (f_c) and dispersion amplitude (δ). For the first hydration shell with the volume fraction ϕ_1 (Fig. 2, please see [3]): $\varepsilon^*_{q1} = \varepsilon_{q\infty1} + \delta_1/\{1 + i(f/f_{c1})\}$, and for the second shell with the volume fraction ϕ_2 : $\varepsilon^*_{q2} = \varepsilon_{q\infty2} + \delta_2/\{1 + i(f/f_{c2})\}$, where both $\varepsilon_{q\infty1}$ and $\varepsilon_{q\infty2}$ are the dielectric constants at a high frequency limit for the inner shell (set to be 5.6 as in our earlier study [4]) and for the outer shell (set here to be 5.6 as for free water [20]), respectively. Then the set of parameters (f_{c1}, δ_1, ϕ_1; f_{c2}, δ_2, ϕ_2) was obtained as described in [3]. Finally, assuming that S1-bound F-actin solution is a one-solute/one-solvent system, the dielectric spectrum of the hydrated solute $\varepsilon^*_q(f)$ with the volume fraction

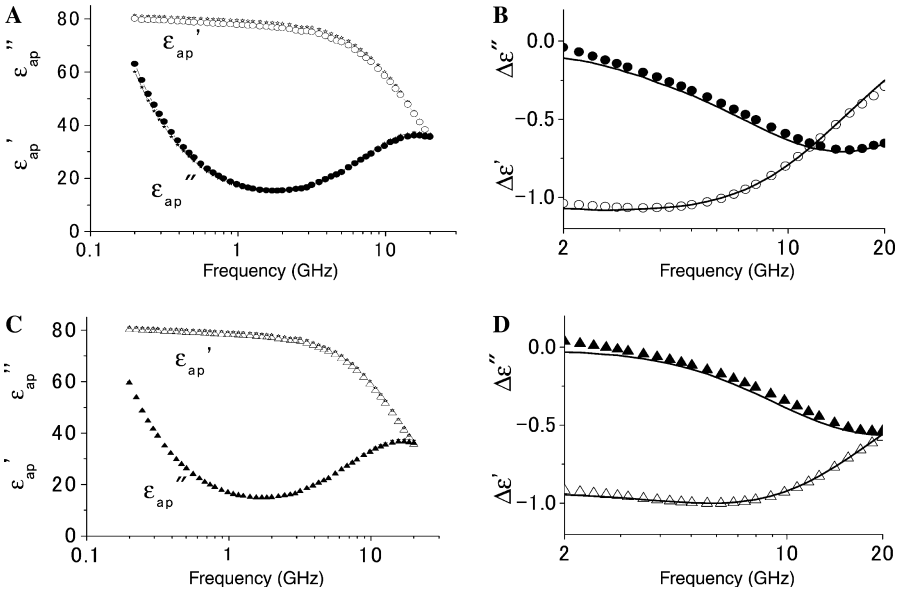

Fig. 1. Dielectric spectra of acto-S1 and S1 solutions. Open and closed symbols refer to the real and imaginary parts of dielectric spectra, respectively (A) Spectra of acto-S1 solution (circles, 16.3 mg/ml) in buffer containing 10 mM Hepes (pH 7.2), 0.2 mM ATP, 0.1 mM $CaCl_2$, 50 mM KCl, and 2 mM $MgCl_2$, and the buffer (stars). (B) Spectra of S1 solution (triangles, 11.5 mg/ml) in buffer containing 10 mM Hepes, 50 mM KCl, and 2 mM $MgCl_2$, and the buffer (stars). (C) Difference dielectric spectrum of acto-S1 (acto-S1 solution vs. buffer) corresponding to (A). Solid lines are the fitting curves. (D) Difference dielectric spectrum of S1 (S1 solution vs. buffer) corresponding to (B). Solid lines are the theoretical curves.

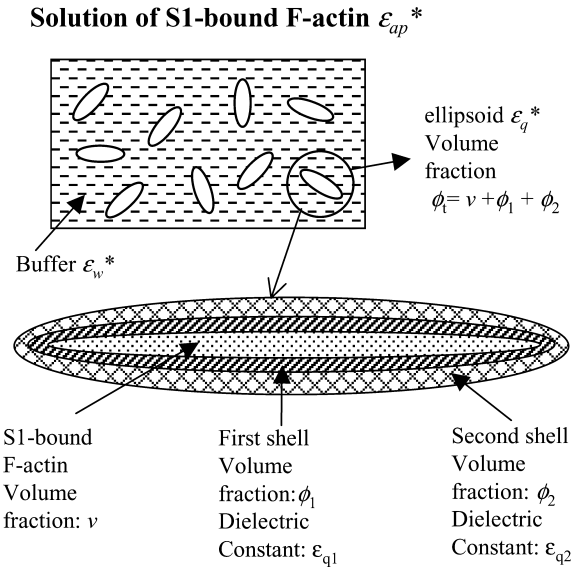

Fig. 2. Double-shelled ellipsoid model of S1-bound F-actin.

$\phi_t = v + \phi_1 + \phi_2$ was calculated by solving the equation of the shelled-ellipsoid model [3,19].

Results and discussion

Actomyosin complex for dielectric spectroscopy

A prerequisite for dielectric measurements is a highly concentrated protein solution (>10 mg/ml). Hence, acto-S1 solutions were prepared using a two-step procedure. Actin was first polymerized by increasing the ionic strength and S1 was then mixed in the presence of 0.2 mM ATP to ensure thorough mixing of these proteins at high concentrations. The ATP added should have been completely hydrolyzed into ADP and inorganic phosphate (P_i) during the pre-incubation period (~1 h) at 20 °C. In the presence of ADP, the interaction between actin and S1 forms a closed set of equilibria (P_i contribution is negligible):

$$\begin{array}{ccc} \mathrm{A+S1+ADP} & \overset{K_1}{\rightleftarrows} & \mathrm{A+S1:ADP} \\ K_3 \quad \downarrow\uparrow & & \downarrow\uparrow \quad K_2 \\ \mathrm{A:S1+ADP} & \underset{K_4}{\rightleftarrows} & \mathrm{A:S1:ADP} \end{array}$$

where A denotes actin, K_1 and K_2 are the equilibrium constants for S1 and acto-S1 to bind ADP, respectively, and K_3 and K_4 are those for the association of proteins. Literature values are available for these K_is [21] to calculate the relative proportion of the four states of S1 (free S1, S1:ADP, A:S1, and A:S1:ADP complexes). This indicates that most of S1 (>98%) would be in the A:S1:ADP state under the experimental conditions used in this study.

Dielectric properties of actomyosin complex

The acto-S1 solution prepared was then introduced into the measuring cell connected to a microwave network analyzer and the dielectric spectrum, ε^*_{ap}, was recorded (Fig. 1A). In the low microwave frequency

region (<2 GHz), the ionic conduction effect was observed for ε''_{ap}, so that analysis of the dielectric spectra was only made for the data in the frequency range $\geqslant$2 GHz and so this is the description referred to unless otherwise stated. The difference in the dielectric properties between the protein solution and the solvent is very small but systematic (Fig. 1A), which can be clearly shown by the difference spectrum, $\Delta\varepsilon_{ap}(f)$ (Fig. 1C). We confirmed that both $\Delta\varepsilon'_{ap}$ and $\Delta\varepsilon''_{ap}$ values calculated at the different frequencies were proportional to the acto-S1 concentration (Fig. 3), indicating that no significant aggregation occurred in our measurements.

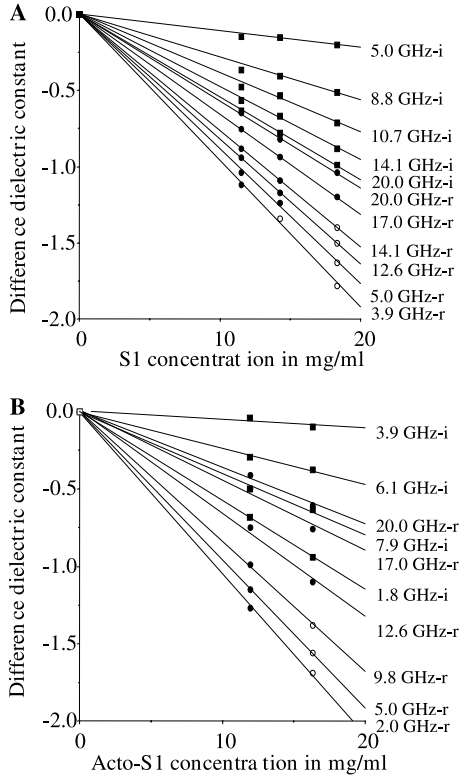

Fig. 3. Linear relation between difference dielectric constant and protein concentration (A) for S1 and (B) for acto-S1. "r" and "i" denote real and imaginary parts of the complex dielectric constant, respectively.

Although the acto-S1 $\Delta\varepsilon(f)$ was very similar to the actin $\Delta\varepsilon(f)$, there were small but significant differences between them (Fig. 4A). Thus, acto-S1 $\Delta\varepsilon''(f)$ is always positioned below actin $\Delta\varepsilon''(f)$ at frequencies >2 GHz, whereas acto-S1 $\Delta\varepsilon'(f)$ outgrows actin $\Delta\varepsilon'(f)$ > 6 GHz, These are the reflections of differences in the dielectric properties of the hydrated acto-S1 and actin, which are illustrated in the calculated dielectric spectra, $\varepsilon_q(f)$s, of hydrated proteins (Fig. 4B. The acto-S1 ε''_q increases steadily with frequencies >2 GHz, while actin ε''_q shows a small, broad hump around 6 GHz and becomes smaller than acto-S1 ε''_q. On the other hand, there are obvious differences between acto-S1 $\varepsilon'_q(f)$ and actin $\varepsilon'_q(f)$. There is a distinctive gap between ε'_q and ε''_q in the high frequency region (>7 GHz) for hydrated actin, which has not been observed for myoglobin or other globular proteins examined so far [3,4]. This suggests that there exists water component having a dielectric relaxation frequency higher than that of bulk water. This component is referred to as hyper-mobile water [3]. Thus, hydrated acto-S1 is qualitatively similar to hydrated actin having a hydration shell of hyper-mobile water, which differs quantitatively from that of actin.

The dielectric data were analyzed as described in Materials and methods. The dielectric parameters, dielectric relaxation frequency ($f_{ci}^{acto\text{-}S1}$), dispersion amplitude ($\delta_i^{acto\text{-}S1}$), and volume fraction of hydrated solute ($\phi_i^{acto\text{-}S1}$) are shown in Table 1. This analysis provided a satisfactory fitting of the curves for the observed difference spectrum of acto-S1 $\Delta\varepsilon(f)$ (Fig. 1C). Compared with the parameters for actin, no significant changes are observed for any parameters in the first hydration component. For the second hydration component, however, a large increase in the volume fraction of hydrated solute ($\phi_2^{acto\text{-}S1}$) is obvious with little changes in $f_{c2}^{acto\text{-}S1}$ and $\delta_2^{acto\text{-}S1}$ values, which is reflected in the spectral difference between acto-S1 and actin (Figs. 4A and B). The dielectric spectra of S1 with bound ADP, $\varepsilon(f)$ (Fig. 1B) and $\Delta\varepsilon(f)$ (Fig. 1D), are similar to those for myoglobin and other globular proteins examined recently [3,4]. For the spectrum of the hydrated solute of S1(ADP), $\varepsilon''_q(f)$ shows a broad but clear peak at

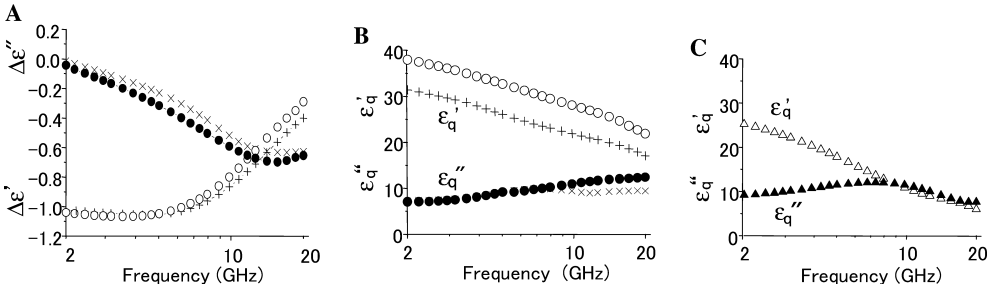

Fig. 4. Difference dielectric spectra of acto-S1 and S1 solutions. Open and closed symbols refer to the real and imaginary parts of dielectric spectra, respectively. Data were normalized to a protein concentration of 10 mg/ml. (A) Difference dielectric spectra of acto-S1(circles) and F-actin (+:real, x:imaginary). (B) Dielectric spectra of hydrated acto-S1and F-actin. Symbols are defined same as in (A). (C) Dielectric spectrum of hydrated S1.

7.5 GHz, and $\varepsilon'_q(f)$ decreased steeply above 4 GHz (Fig. 4C). Both these features are quite distinct from the acto-S1 spectra. The dielectric spectra of S1(ADP) solution can be well described by a single-shelled sphere model for globular proteins [4]. Both the S1(ADP) $\Delta\varepsilon'(f)$ and $\Delta\varepsilon''(f)$ could be well fitted by theoretical curves from 2 to 20 GHz (Fig. 1D). These results indicate that there is no relaxation component with a high f_c for the hydration of S1(ADP). Essentially the same result was obtained for ADP-free S1 (Table 1). Nevertheless, S1 affects the double hydration shell of F-actin (Table 1).

First, the volume fraction of the first hydration shell is considered. Assuming that the acto-S1 is formed from the simple binding of actin and S1 without involving reorganization of their hydration shells, the value of $\phi_1^{\text{acto-S1}}$ can be calculated as $\phi_1^{\text{acto-S1}} = (13\phi_1^{\text{actin}} + 2\phi_1^{\text{S1}})/13$. The value obtained is in good agreement with the observed value, suggesting that no appreciable change is induced in the volume of the first hydration shell upon acto-S1 formation. For the acto-S1 complex it has been estimated that a decrease in ASA (accessible surface area) of $\sim$11 nm^2 would be as large as $\sim$4% for S1 and $\sim$10% for actin [22,23], which then should be accompanied by a displacement of $\sim$100 water molecules. On the basis of an actin unit, this would only correspond to a decrease of less than 3%, which is within the experimental error in the present study.

For the second hydration shell, the result of $\phi_2^{\text{acto-S1}} > \phi_2^{\text{actin}}$ indicates that on the G-actin unit basis, there is a large increase ($\sim$30%) of the volume fraction of the hyper-mobile water when F-actin binds to S1 which has no such water. If only the G-actin unit was considered to directly interact with S1, then ϕ_2 would be several times as large as of its molecular volume.

Structure and hydration of actomyosin complex

Theories worked out for the water-structure breaking effect of alkali metal and halide ions [24,25] suggest that the effect can be attributed to the surface electric field around the solute and the balance of strength between water–solute electrostatic interaction and water–water hydrogen bond. Using our direct method of dielectric spectroscopy of the equivalence of effects of water-structure breaking and induction of hyper-mobile water [3], along with a reported calculation of the electrostatic potential for F-actin [26], we have attempted to explain by analogy how hyper-mobile water is induced around F-actin. F-actin can be seen as a double helix of bulbs of negative potential extending into the solvent. There is a region in the vicinity of the surface of actin sub-domain I (SD-I), where the estimated strength of the electric field is in the range 10–50 MV/m considering the net charge ($\sim -8e$) and the radius $\sim$1μm of SD-I. The strength is comparable to that around I^-, a typical water-structure breaking ion. The hydrogen-bonded network of water may be affected in this area in such a way that some of the bonds are short-lived and thereby enable water molecules to become more mobile. The next question is then how the binding of S1(ADP) could affect this increase in volume. In general, the strength of the electric field decays rather sharply as a function of distance from the charged surface due to the Debye screening effect, which would be damped down within 1 nm from the surface. Thus, there are only two possible ways to increase the volume of hyper-mobile water: (1) increase the total surface area of the appropriate charge or (2) modulate the strength of the electric field specifically generated by actin.

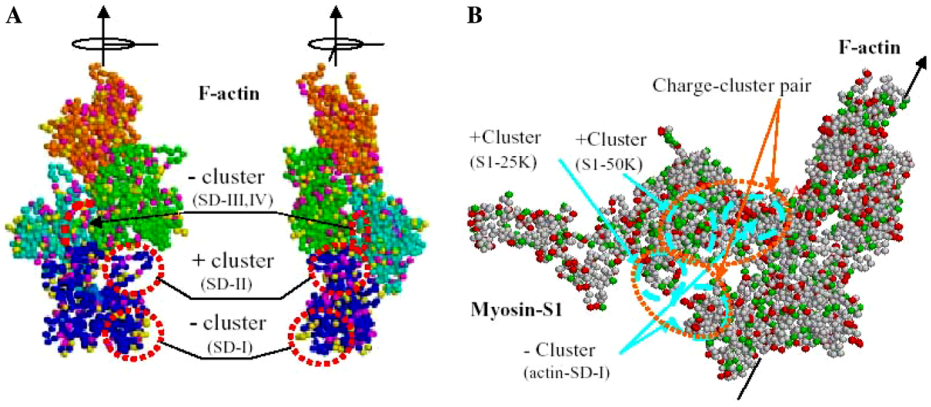

Fig. 5. Surface charge distribution of the actin and acto-S1 complex. The images are drawn based on the theoretical model composed of α-carbons from PDB1ALM. (A) Charge clusters on the surface of an F-actin segment. Images were obtained by removing the S1 portion from the original image (shown in B). The image on the right is the same as the one on the left but rotated 90° around its axis. Each actin monomer unit is displayed with different colors with the color codes for charged residues as follows: magenta, positively charged; yellow, negatively charged. Red dashed circles show the charged clusters. (B) Charge distribution of acto-S1 drawn using the original model of PDB1ALM. Green and red marks indicate positive and negative charges, respectively. Note that a positively charged cluster in the lower 50K-domain of S1 faces the negatively charged cluster of the sub-domain-I (SD-I) of actin, while positive charges of the 25K-domain of S1 face the negative charge of SD-I of the actin adjacent unit (barbed-end side) which directly interacts with S1. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this paper.)

The S1-increased ASA itself will not make any contribution to the volume increase of hyper-mobile water, because no such water exists around S1. It cannot be ruled out, however, that a certain binding-induced reorganization of the charge density occurs somewhere on the surface of S1 and/or actin, which results in a net increase of hyper-mobile water. The second possibility is a dilatation of the effective region in the decaying electric field around the surface of actin SD-I. It seems that in the 3-D atomic structure of acto-S1 complex [27,28], the lower 50K domain of S1 bound to a G-actin unit and the SD-I of the latter locks up a three dimensional half-closed space (pocket) (Fig. 5). A part of the S1 surface is rich in positive charge which faces the negatively charged actin surface. This would in effect generate an electric field with an appropriate strength to accommodate a large amount of hyper-mobile water. Alternatively, as predicted by simulation [29] and as observed [30] for some other actin-binding proteins, the S1 could induce a change of the topological position for the SD-I of G-actin unit by directly interacting with S1 relative to the SD-II of the adjacent unit along the F-actin axis. Unlike the SD-I, SD-II is rich in positive charge, so that the electric field formed in between could be modulated in a similar way to the case of the actin/S1 sub-domain interaction.

Implication in chemo-mechanical energy conversion

As previously predicted [3] and confirmed here, a quantitative change of the hyper-mobile component occurs upon S1 binding. Such dynamic change of actin hydration would provide a novel approach to our understanding of the chemo-mechanical energy conversion by actomyosin system. Since the high relaxation frequency of molecular orientation of water reflects loosening of cooperative hydrogen-bond network among water molecules to lower the viscosity [31,32], the present result indicates that the solvent space around S1 bound to F-actin is asymmetric in viscosity (Fig. 7 of [3]). Consequently, once this myosin motor domain goes into the weak-binding state (low affinity for actin) by binding and splitting ATP, its center of mass tends to move toward the direction of lower viscosity by Brownian motion along the filament axis under thermal agitation. This viscosity-based Brownian ratchet model may provide a plausible answer to the key issue of "symmetry breaking" common to any of the models of chemo-mechanical energy conversion [33,34].

The results show us that water is by no means just a simple milieu for proteins to fold and function, but rather is an active agent that can change its dynamic properties depending on conformation of the proteins. In particular for interacting proteins, the water provides a go-between by changing its dynamic properties in response to protein structures, which in return affects protein dynamics.

Acknowledgments

We thank K. Mihashi, Y. Maeda, J.P. Gong, Y. Osada, H. Higuchi, and Y. Yasunaga for discussions, and J. West for reading the manuscript. This work was supported by Grants-in-Aid for Scientific Research on Priority Areas (No. 16041203) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

[1] R.G. Bryant, The dynamics of water–protein interactions, Annu. Rev. Biophys. Biomol. Struct. 25 (1996) 29–53.
[2] B. Halle, V.P. Denisov, Magnetic relaxation dispersion studies of biomolecular solutions, Methods Enzymol. 338 (2001) 178–201.
[3] S.R. Kabir, K. Yokoyama, K. Mihashi, T. Kodama, M. Suzuki, A hyper-mobile state is induced among water molecules neighbouring actin filaments, Biophys. J. 85 (2003) 3154–3161.
[4] K. Yokoyama, T. Kamei, H. Minami, M. Suzuki, Hydration study of globular proteins by microwave dielectric spectroscopy, J. Phys. Chem. B. 105 (2001) 12622–12627.
[5] M. Suzuki, J. Shigematsu, Y. Fukunishi, T. Kodama, Hydrophobic hydration analysis on amino acid solutions by the microwave dielectric method, J. Phys. Chem. B 101 (1997) 3839–3845.
[6] R. Buchner, J. Barthel, J. Stauber, The dielectric relaxation of water between 0 and 35 °C, Chem. Phys. Lett. 306 (1999) 57–63.
[7] G.W. Robinson, S.B. Zhu, S. Singh, M.W. Evans, Water in BiologyWorld Scientific Series in Contemporary Chemical Physics, vol. 9, World Scientific, Singapore, 1996.
[8] M.C. Bellissent-Funel, Hydration Process in BiologyNATO Science Series A, vol. 305, OS Press, Amsterdam, 1999.
[9] C. Mattos, Protein–water interactions in a dynamic world, Trends Biochem. Sci. 27 (2002) 203.
[10] T. Yamada, 1H-nuclear magnetic resonance evidence for actomyosin-dependent structural changes of the intracellular water of frog skeletal muscle fiber, Biochim. Biophys. Acta 1379 (1998) 224–232.
[11] P. Sheterline, J. Clayton, J.C. Sparrow, Actin, fourth ed., Oxford University, Oxford, 1998.
[12] R. Cooke, Actomyosin interaction in striated muscle, Physiol. Rev. 77 (1997) 671–697.
[13] M.A. Geeves, K.C. Holmes, Structural mechanism of muscle contraction, Annu. Rev. Biochem. 68 (1999) 687–728.
[14] J.A. Spudich, S. Watt, The regulation of rabbit skeletal muscle contraction. 1. Biochemical studies of the interaction of the tropomyosin–troponin complex with actin and the proteolytic fragments of myosin, J. Biol. Chem. 246 (1971) 4866–4871.
[15] K.M. Nauss, S. Kitagawa, J. Gergely, Pyrophosphate binding to and adenosine triphosphatase activity of myosin and its proteolytic fragments. Implications for the substructure of myosin, J. Biol. Chem 244 (1969) 755–765.
[16] S.S. Margossian, S. Lowey, Preparation of myosin and its subfragments from rabbit skeletal muscle, Methods Enzymol. 85 (Pt. B) (1982) 55–71.
[17] A.G. Weeds, R.S. Taylor, Separation of subfragment-1 isoenzymes from rabbit skeletal muscle myosin, Nature 257 (1975) 54–56.

[18] T. Kodama, K. Fukui, K. Kometani, The initial phosphate burst in ATP hydrolysis by myosin and subfragment-1 as studied by a modified malachite green method for determination of inorganic phosphate, J. Biochem. 99 (1986) 1465–1472.
[19] K. Asami, T. Hanai, N. Koizumi, Dielectric analysis of *Escherichia coli* suspensions in the light of the theory of interfacial polarization, Biophys. J. 31 (1980) 215–228.
[20] U. Kaatze, On the existence of bound water in biological systems as probed by dielectric spectroscopy, Phys. Med. Biol. 35 (1990) 1663–1681.
[21] S.J. Smith, H.D. White, R.C. Woledge, Microcalorimetric measurement of the enthalpy of binding of rabbit skeletal myosin subfragment 1 and heavy meromyosin to F-actin, J. Biol. Chem. 25 (1984) 10303–10308.
[22] M. Suzuki, J. Shigematsu, Y. Fukunishi, Y. Harada, T. Kodama, T. Yanagida, Coupling of protein surface hydrophobicity change to ATP hydrolysis by myosin motor domain, Biophys. J. 72 (1997) 18–23.
[23] S. Highsmith, K. Duignan, R. Cooke, J. Cohen, Osmotic pressure probe of actin–myosin hydration changes during ATP hydrolysis, Biophys. J. 70 (1996) 2830–2837.
[24] B. Hribar, T.N. Southall, V. Vlachy, K.A. Dill, How ions affect the structure of water, J. Am. Chem. Soc. 124 (2002) 12302–12311.
[25] T. Imai, H. Nomura, M. Kinoshita, F. Hirata, Partial molar volume and compressibility of alkali-halide ions in aqueous solution: Hydration shell analysis with an integral equation theory of molecular liquids, J. Phys. Chem. B. 106 (2002) 7308–7314.
[26] I.V. Ouporov, H.R. Knull, K.A. Thomasson, Brownian dynamics simulations of interactions between aldolase and G- or F-actin, Biophys. J. 76 (1999) 17–27.
[27] M. Lorenz, D. Popp, K.C. Holmes, Refinement of the F-actin model against X-ray fiber diffraction data by the use of a directed mutation algorithm, J. Mol. Biol. 234 (1993) 826–836.
[28] M.C. Reedy, Visualizing myosin's power stroke in muscle contraction, J. Cell Sci. 113 (2000) 3551–3562.
[29] R. Page, U. Lindberg, C.E. Schutt, Domain motions in actin, J. Mol. Biol. 280 (1998) 463–474.
[30] A. Orlova, V.E. Galkin, M.S. VanLoock, E. Kim, A. Shvetsov, E. Reisler, E.H. Egelman, Probing the structure of F-actin: cross-links constrain atomic models and modify actin dynamics, J. Mol. Biol. 312 (2001) 95–106.
[31] I.L. Fabelinskiĭ, Macroscopic and molecular shear viscosity, Physics-Usppekhi 40 (1997) 689–700.
[32] V.A. Bloomfield, Separations and Hydrodynamics, Biophysics textbook online. Avialable from: <http://www.biophysics.org/btol/supramol.htmlbt.pdf>, Biophysical Soc., 2002.
[33] R.D. Vale, F. Oosawa, Protein motors and Maxwell's demons: does mechanochemical transduction involve a thermal ratchet?, Adv. Biophys. 26 (1990) 97–134.
[34] R.D. Astumian, Scientific American 285 (2001) 56–64.