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Effects of urea and guanidine hydrochloride on the sliding movement of actin filaments with ATP hydrolysis by myosin molecules

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To evaluate the role of the hydration layer on the protein surface of actomyosin, we compared the effects of urea and guanidine-HCl on the sliding velocities and ATPase activities of the actin-heavy meromyosin (HMM) system. Both chemicals denature proteins, but only urea perturbs the hydration layer. Both the sliding velocity of actin filaments and actin-activated ATPase activity decreased with increasing urea concentrations. The sliding movement was completely inhibited at 1.0 M urea, while actin filaments were bound to HMM molecules fixed on the glass surface. Guanidine-HCl (0–0.05 M) drastically decreased both the sliding velocity and ATPase activation of acto-HMM complexes. Under this condition, actin filaments almost detached from HMM molecules. In contrast, the ATPase activity of HMM without actin filaments was almost independent of urea concentrations <1.0 M and guanidine-HCl concentrations <0.05 M. An increase in urea concentrations up to 2.0 M partly induced changes in the ternary structure of HMM molecules, while the actin filaments were stable in this concentration range. Hydration changes around such actomyosin complexes may alter both the stability of part of the myosin molecules, and the affinity for force transmission between actin filaments and myosin heads.

Keywords: actomyosin/ATP hydrolysis/denaturant/hydration/motility.

Abbreviations: ATP, adenosine-5′-triphosphate; bis-ANS, 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulfonic acid; DTT, dithiothreitol; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HMM, heavy meromyosin; PCA, perchloric acid; Pi, inorganic phosphate.

Myosin, which is one of the major ATPases in eukaryotic cells, transforms the released energy from ATP hydrolysis into mechanical energy that drives the sliding movement of an actin filament. The energy transduction by actomyosin molecules may also be associated with hydration changes, which occur on the protein surface, and with properties of the interplay of water molecules (*1*, *2*). Regarding the water molecules around the actomyosin complex, Kabir *et al*. (*3*) observed hypermobile water, which facilitates faster motion compared to bulk water, going beyond the hydration layer of actin filaments. Furthermore, they reported that the amount of hypermobile water around the filaments increased when myosin heads are bound to them, indicating a likelihood for modulating water properties to accommodate the interaction with the actomyosin complex (*4*). When the entropy change of the water layer occurs around actin filaments, it is possible for the motor protein, such as myosin II of skeletal muscle, to travel faster over a longer distance beyond several actin constituents along the filament during 1 cycle of ATP hydrolysis (*5*). However, there is little evidence for a connection between those changes in the water properties and the accompanying mechanochemical transduction.

In the context of water properties, there have been many investigations using various techniques that focus on the nature of hydrogen bonding around solutes, including metal ions, sugar, urea, protein molecules, and many others (*6–12*). Nonetheless, it still remains unclear how these solutes influence the function of nearby macromolecules. In typical studies, the urea solute is used as a denaturant for unfolding proteins at a high concentration (*e.g.* above 6.0 M). Recently, the denaturation mechanism of proteins in the presence of high urea concentrations was explicated by a simulation using molecular dynamics (*6*). Urea molecules have little effect on the network of bulk water molecules. However, they interact directly with the backbone and side chains of proteins, resulting in the breaking of intra-backbone hydrogen bonds. Other experiments also support that urea does not strongly change the nature of bulk water but influences the hydration layer of proteins by weakening hydrogen bonds in the layer (*7–10*).

In the present article, we focused on the contribution of water molecules to the interaction between actin and myosin molecules. For the stated objective, we employed urea molecules as an agent for perturbing the hydrogen bonds of the water molecules around the actomyosin complex. To test whether the observed changes in the actomyosin interaction is caused by a direct effect of perturbed hydrogen bonds or an indirect effect via partial denaturation, we also examined the effects of guanidine-HCl, a potent denaturant that does not perturb hydrogen bonds (*13*). We directly

measured the sliding velocity of fluorescently labeled actin filaments on heavy meromyosin (HMM) molecules. The measurement was performed using an *in vitro* motility assay. The ATPase activity of the acto-HMM complex was measured using a malachite green method for the determination of inorganic phosphate (Pi) released during ATP hydrolysis. Both the sliding velocity and actin-activated ATPase activity of HMM were significantly suppressed in the presence of urea, while ATPase activity in the absence of actin filaments remained rather indifferent to the presence of the solute. Compared to urea, guanidine-HCl was more effective in decreasing both motile activity and ATPase activation.

Materials and Methods

Reagents and proteins

Tetramethylrhodamine-phalloidin and 4,4′-dianilino-1,1′-binaphthyl-5,5′-disulphonic acid (*bis*-ANS) were purchased from Sigma-Aldrich. Other chemicals were purchased from Nacalai Tesque (Kyoto) and were of special reagent grade. Actin and myosin were prepared from rabbit skeletal muscle. The actin monomers were purified according to the method of Spudich and Watt (*14*). The myosin molecules were purified using the method of Perry (*15*) and then were digested with α-chymotrypsin for the preparation of HMM molecules, which have two identical catalytic heads and are freely soluble in aqueous solution.

Measurements of fluorescence

Fluorescence measurements of actin filaments stabilized by phalloidin and of HMM molecules were performed with a spectrofluorometer (Hitachi, F-2500). Emission spectra of intrinsic fluorescence derived from tryptophan residues were measured at various concentrations of urea or guanidine-HCl in solutions containing 25 mM KCl, 25 mM HEPES (pH 7.4) and 4 mM $MgCl_2$, at a protein concentrations of 0.05 mg/ml at 25°C. Individual samples were prepared at each concentration of urea or guanidine-HCl with an incubation time of 10 min after mixing proteins and solvents. To avoid unnecessary fluorescence decay, the window of the excitation light was closed until the measurement was started. Excitation was set at 280 nm (band pass 10 nm), and the emission was monitored between 300 and 400 nm. The red shift of the spectrum was evaluated by calculating the spectral center of mass from 300 to 400 nm. The spectral area was calculated as the sum of fluorescence intensities in the above wavelength range.

Other fluorescence measurements were carried out using *bis*-ANS as a fluorescent probe responding to hydrophobic environments. The *bis*-ANS dye was added to the solution at the same conditions used for intrinsic fluorescence measurements. After all additions, the concentration of *bis*-ANS and proteins were 0.002 mg/ml and 0.05 mg/ml, respectively. Excitation was set at 360 nm (band pass 10 nm), and the emission was monitored between 420 and 600 nm. The spectral area was calculated as the sum of fluorescence intensities in the wavelength range mentioned above.

Measurement of sliding velocity

The prepared actin filaments were labeled with tetramethylrhodamine-phalloidin. HMM molecules were fixed on the collodion-coated surface of glass slides (Matsunami, No. 1, 24 × 50 mm) by a perfusion of HMM solution [0.05 mg/ml HMM, 25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 0.5% 2-mercaptoethanol] between the slide and the cover glass (Matsunami, No. 1, 18 × 18 mm) with 0.1 mm separation. Sixty seconds after perfusion, the solution was replaced with BSA solution [3 mg/ml bovine serum albumin, 25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 0.5% 2-mercaptoethanol], so that unbound HMM molecules were removed. Subsequently, the slide was perfused with 1 μg/ml of labelled actin solution. Immediately after replacement by ATP solution [25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 1 mM ATP, 0.5% 2-mercaptoethanol, 3 mg/ml glucose, 0.1 mg/ml glucose oxidase, 0.02 mg/ml catalase] containing urea or guanidine-HCl at various concentrations, the sliding movement of actin filaments was observed under a fluorescence microscope (Nikon, Diaphoto-TMD, objective DIC 100× oil) with the aid of fluorescent equipment (Nikon, TMD-EF2) and optical interference filters optimized for rhodamine dyes (Omega, XF101-2). Each experiment was done within approximately 10 min at 27°C after ATP solution was added.

The fluorescent images from a highly sensitive camera (Hamamatsu, C2400-08) were recorded on a computer (Apple Co., Power Mac G3) through a video grabber board (Scion Co., LG-3). The sliding velocity of the actin filaments was determined by measuring the moving distance at an interval of every 0.5 s with the help of image analysis software (NIH, ImageJ). The sliding velocity of actin filaments was determined by averaging 100 independent samples in each case. Spacing between the nearest-neighbour pixels in the image was 0.083 μm.

Measurement of ATPase activity

ATP hydrolysis was monitored by measuring the concentration of Pi using a malachite green method (*16*). Malachite green reagent consisted of 0.06 g malachite green, 2.46 g sodium molybdate, 0.1 g Triton-X in 200 ml of 1 M HCl. Just before use, this reagent was mixed with an equal volume of 0.3 M PCA.

The ATPase reaction was initiated by adding 50 μl of 2 mg/ml HMM to 950 μl of F-actin solution [0.3 mg/ml F-actin, 25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 1 mM ATP and variable concentrations of urea or guanidine-HCl]. The reaction was performed at 25°C. At 5 min intervals, 20 μl of sample was taken from the reaction mixture, and then 2 ml of malachite green reagent was added for termination and coloration. Immediately after sampling, 200 μl of 34% sodium citrate solution (pH 8) was added, and the reaction was agitated for few seconds. After incubation for 13 min at 25°C, the absorbance of the sample was measured at 650 nm by spectrometry. The ATPase activity was counted as the molar ratio of Pi release to HMM molecule per second.

Results

Effects of urea and guanidine-HCl on the ternary structure of actin filaments and HMM molecules

To evaluate urea-induced unfolding of actin filaments and HMM molecules, the change in the intrinsic fluorescence spectrum was measured in the presence of urea or guanidine-HCl. In general, the wavelength maximizing the fluorescence intensity of tryptophan residues has a tendency to shift toward the longer wavelength range, when the residues in the hydrophobic region are exposed to polar environments. The slight red shift of the intrinsic fluorescence of actin filaments that was stabilized by phalloidin occurred at concentrations of urea <2.0 M (Fig. 1A). In the presence of HMM, the spectral shift was gradually enhanced as the urea concentration increased (Fig. 1B). Then, guanidine-HCl was found to induce a larger spectral shift than did urea. In addition, the spectral area of the intrinsic fluorescence intensity of HMM was slightly increased by 8%, when the urea concentration increased from 0 to 1.0 M (Fig. 1C).

The other fluorescence measurements were carried out using *bis*-ANS, whose fluorescence intensity is enhanced in response to binding to hydrophobic regions, such as the catalytic ATP-binding site of the myosin head. Figure 2 demonstrates the enhancement of the fluorescence intensity of *bis*-ANS bound to actin or HMM in the presence of urea or guanidine-HCl. In the presence of actin filaments, the fluorescence intensity of *bis*-ANS decreased slightly as the urea concentration increased (Fig. 2A). Guanidine-HCl at concentrations up to 2.0 M induced an ~4-fold

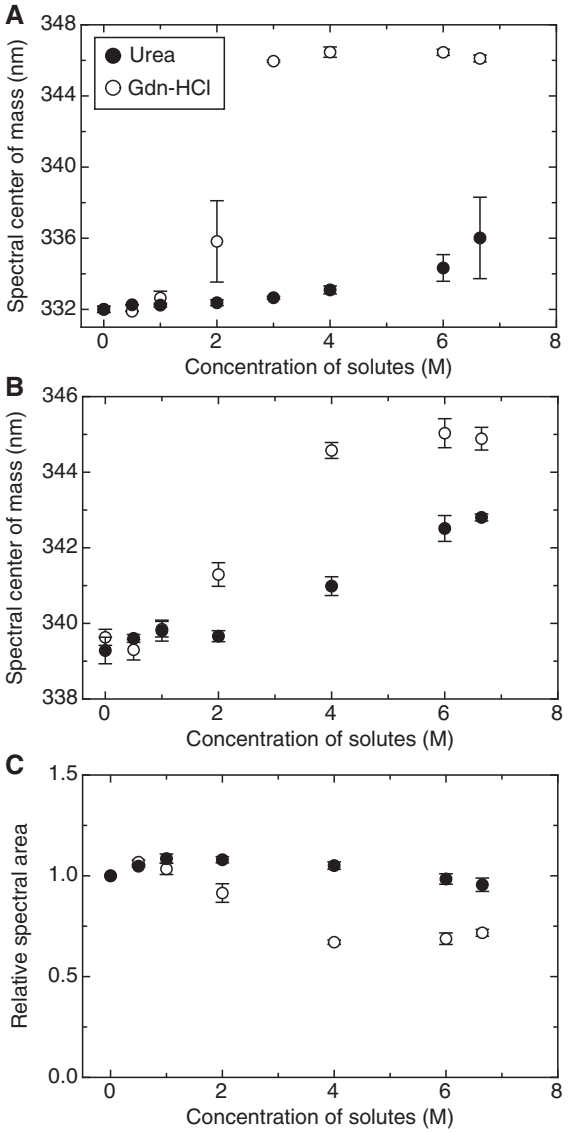

Fig. 1 Spectral shift of the intrinsic fluorescence of actin filaments (A) and HMM molecules (B) by the presence of urea or guanidine-HCl. The spectral area of the intrinsic fluorescence of HMM molecules is shown in (C). Spectral areas for each condition were normalized by the original area in the absence of solutes. Filled and open symbols represent the presence of urea and guanidine-HCl, respectively. The excitation was set at 280 nm, and the emission was monitored between 300 and 400 nm. Error bars indicate standard deviations estimated from three independent experiments under similar conditions.

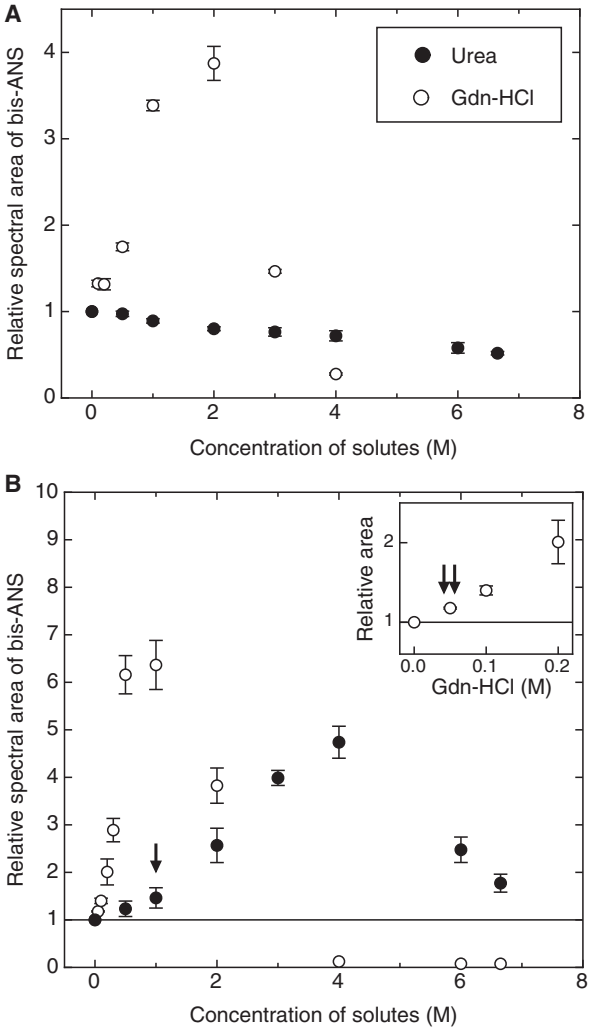

Fig. 2 Spectral area of the fluorescence of *bis*-ANS bound to actin filaments (A) and HMM molecules (B). Filled and open symbols represent the presence of urea and guanidine-HCl, respectively. Inset in (B): the spectral area in guanidine-HCl at concentrations from 0 to 0.2 M. The excitation was set at 360 nm, and the emission was monitored between 420 and 600 nm. Spectral areas for each condition were normalized by the original area in the absence of solutes. Error bars indicate the standard deviations estimated from 3 independent experiments under similar conditions. In the case of HMM molecules (B), 1.0 M urea and 0.05 M guanidine-HCl induced a 1.5-fold increase in fluorescence intensity (indicated by a single arrow) and a 1.2-fold increase in fluorescence intensity (indicated by double arrows), respectively.

increase in the fluorescence intensity of *bis*-ANS, although fluorescence intensity decreased >2.0 M guanidine-HCl. In the presence of HMM, the fluorescence intensity of *bis*-ANS gradually increased up to 4.5-fold as the urea concentration increased from 0 to 4.0 M and decreased with further increase in urea concentrations (Fig. 2B). Guanidine-HCl between 0 and 1.0 M induced a 6.5-fold increase in the fluorescence intensity of *bis*-ANS with HMM. A possible explanation for the enhanced fluorescence intensity due to these solutes is that the hydrophobic region is easily accessible to the bis-ANS probe, because of unstable formations induced by the solutes. The decrease in intensity at the higher concentration of the solutes indicated a disruption of the ternary structure of the hydrophobic regions. Guanidine-HCl was more effective at both enhancing the fluorescence intensity of *bis*-ANS and the red shift of intrinsic fluorescence compared with urea.

Stability of actin filaments in the presence of urea and guanidine-HCl

We examined the extent to which urea and guanidine-HCl may affect the formation of actin filaments. First, actin monomers (67 μg/ml) were completely polymerized into filaments with tetramethylrhodamine-phalloidin in solution A

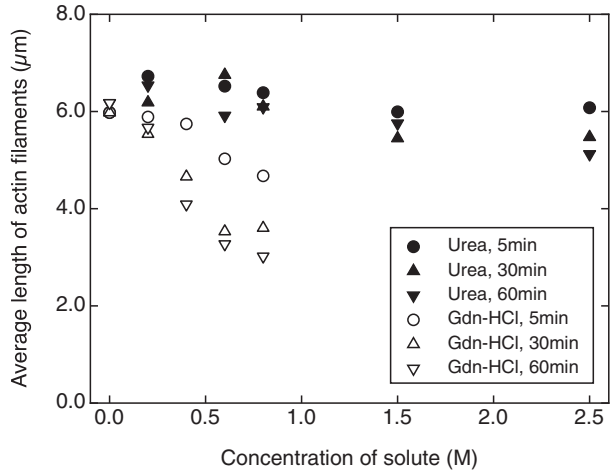

Fig. 3 Average length of actin filaments in the presence of urea (filled symbols) and in the presence of guanidine-HCl (open symbols). For each condition, the length of 200 filaments was directly measured under a fluorescence microscope. After addition of solutes to the actin solution, the following incubation times were tested: 5 min (circles), 30 min (triangles) and 60 min (inverted triangles).

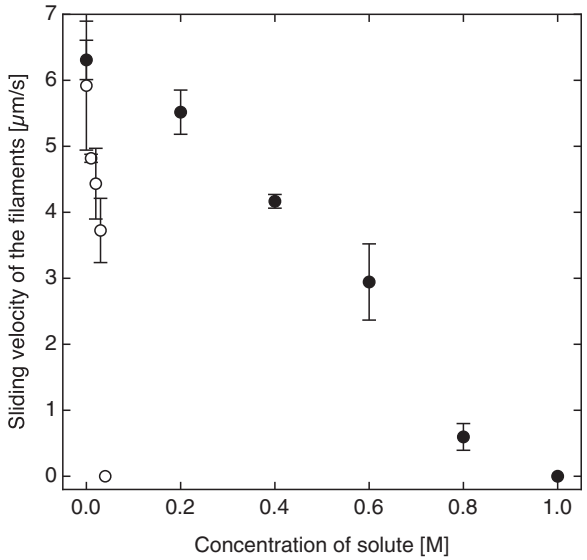

Fig. 4 Sliding velocities of actin filaments along HMM molecules in the presence of urea (filled circles) and in the presence of guanidine-HCl (open circles). Error bars indicate the standard deviations estimated from three independent experiments under similar conditions. For each experiment, the results from 100 filaments were averaged.

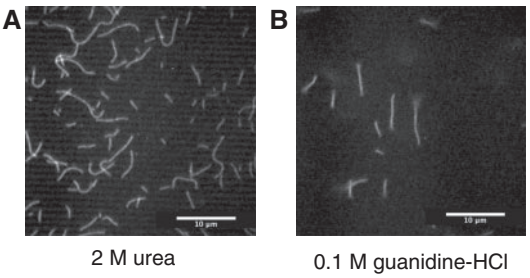

Fig. 5 Fluorescent images of actin filaments on HMM molecules in the presence of 2 M urea (A) and in the presence of 0.1 M guanidine-HCl (B). Other conditions that applied to both cases were 25 mM KCl, 25 mM imidazole–HCl (pH 7.4), 4 mM $MgCl_2$, 1 mM ATP, 0.5% 2-mercaptoethanol, 3 mg/ml glucose, 0.02 mg/ml catalase and 0.1 mg/ml glucose oxidase.

[25 mM KCl, 25 mM imidazole-HCl (pH 7.4), 4 mM $MgCl_2$, 1 mM ATP, 1mM DTT]. Just before the examination, either urea or guanidine-HCl was added to the actin solution (final actin concentration of 1 μg/ml) at various concentrations. Under a microscope, the length of the actin filaments was directly measured after incubation of the solute and actin filaments. Figure 3 shows the average length of actin filaments in the presence of urea and in the presence of guanidine-HCl at various incubation times. In the presence of urea, no change of filament length was observed in the concentration range examined. On the other hand, in the presence of guanidine-HCl, which is ionic, the length of filaments decreased with increasing concentrations and incubation times, indicating that guanidine-HCl induced instability in the filaments above 0.5 M. As a matter of fact, the actin filaments we used remained stable below 2.5 M of urea.

Influence on the sliding movement of actin filaments

A motility assay for the sliding movement of actin filaments along HMM molecules associated with ATP hydrolysis was performed under a microscope. Figure 4 shows the dependence of the sliding velocity on the concentration of solutes. The sliding velocity of actin filaments gradually decreased from 6 to 0 μm/s as the urea concentration increased up to 1 M. Most of the actin filaments, which failed to move, were still bound to the HMM molecules fixed to the glass surface and exhibited a Brownian motion to a lesser extent (Fig. 5A). The presence of 0.05 M guanidine-HCl drastically suppressed the sliding movement of the filaments, which were almost detached from HMM molecules (Fig. 5B).

Further, we examined the reversibility of this effect in the motility assay system. After the suppression conditions were first implemented, urea or guanidine-HCl solutes were then removed from this system by exchanging the solution for one without solutes. For both experiments with urea or guanidine-HCl, the sliding movements of the actin filaments almost recovered to the original levels.

Influence on ATPase activity

ATPase activity of HMM and its activation by actin filaments were examined in solution using a malachite green method. In the presence of either urea or guanidine-HCl, HMM-ATPase activities without actin filaments were independent of the solute concentration and $\sim 0.07\,s^{-1}$. In contrast, HMM-ATPase activity in the presence of actin filaments gradually decreased from 1.65 to $0.33\,s^{-1}$ as the urea concentration increased from 0 to 1.0 M (Fig. 6A). This activity was also decreased with the increase in the concentration of guanidine-HCl from 0 to 0.05 M (Fig. 6B).

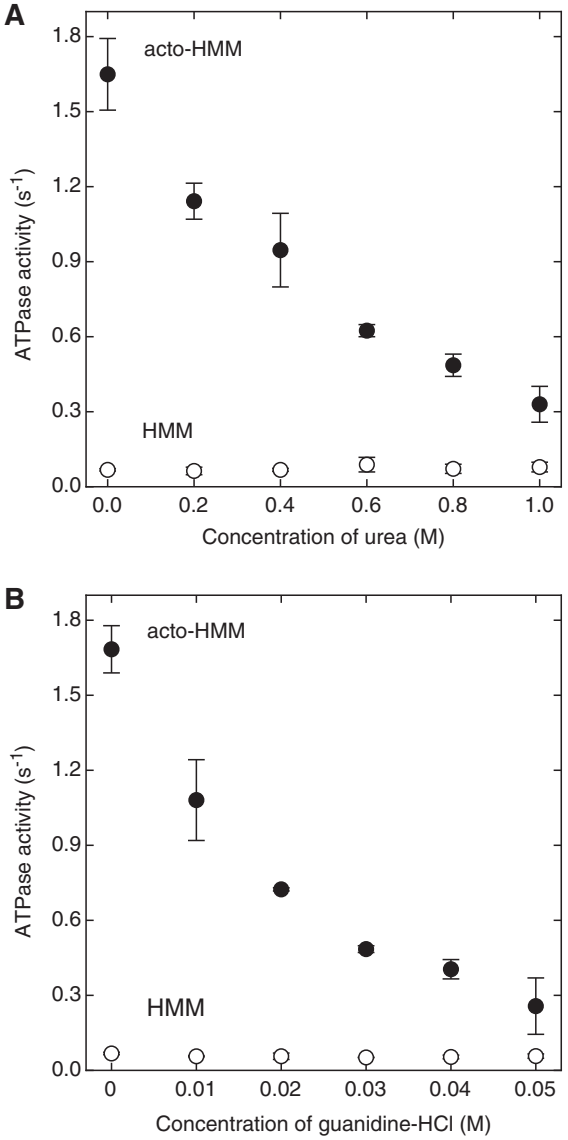

Fig. 6 HMM ATPase activities in the presence of urea (A) and in the presence of guanidine-HCl (B). Filled and open symbols correspond to ATPase activity with and without actin filaments, respectively. Error bars indicate the standard deviations estimated from three independent experiments under similar conditions.

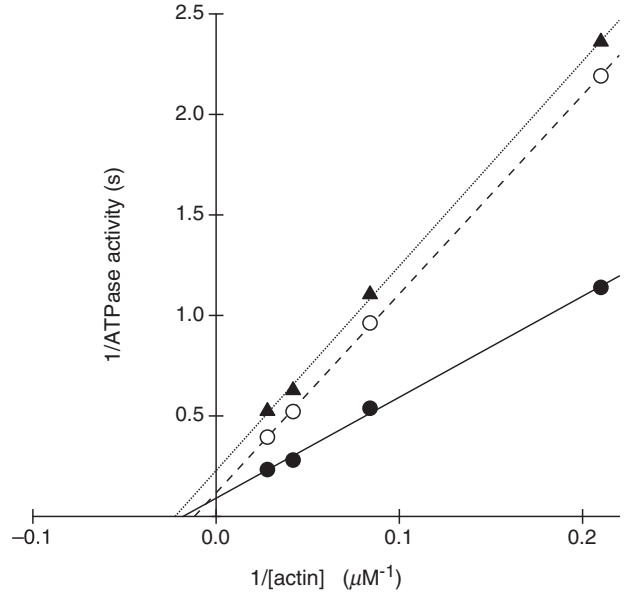

Fig. 7 Dependence of ATPase activities on actin concentration in the presence of 0.5 M urea (open circles) and in the presence of 0.025 M guanidine-HCl (filled triangles). The filled circles denote the absence of both solutes. The relationship between actin concentration and ATPase activity was represented by the double reciprocal plot. Data were the average of three independent experiments. Maximum activities at 0 M, 0.5 M urea and 0.025 M guanidine-HCl were estimated to be 11 s^{-1}, 8.5 s^{-1}, and 4.2 s^{-1}, respectively. Km values at 0 M, 0.5 M urea and 0.025 M guanidine-HCl were 55 μM, 83 μM and 42 μM, respectively.

It was also confirmed that the contribution of urea to the actin-activated ATPase activity was reversible. In fact, when the acto-HMM complex, which was first incubated in a solution of 1 M urea for 60 min, was transferred into the diluted solution of 0.5 M urea, the measured ATPase activity was almost the same strength as that of the samples initially prepared in 0.5 M urea.

To estimate the kinetic parameter Km and the maximum ATPase activity, the effect of actin concentrations on the activated ATPase activity was examined (Fig. 7). In 0.5 M urea solution, the maximum activity decreased to ~75% of the activity in the absence of urea, while the value of the K_m increased up to ~150%. The present observations suggested that the affinity between actin filaments and myosin heads may be weakened in the presence of urea. In contrast, in a solution of 0.025 M guanidine-HCl, the maximum activity decreased to 40% of the original levels in the absence of guanidine-HCl, while the value of the K_m decreased only slightly.

Comparison between the sliding velocity and the ATPase activity

Because the sliding activity of the actomyosin complex in general seems to be coupled with ATPase activity, the solute effect on the sliding velocity was compared to that on the ATPase activity (Fig. 8). In this situation, actin-activated ATPase activity was evaluated as the difference between the HMM ATPase activity with and without actin filaments. In the presence of urea, the tendency for the sliding velocity to decrease as the urea concentration increased was almost similar to that for the ATPase activity. However, it was observed that in the presence of 0.8 M urea, ATPase activation was enhanced as much as 26%, while the sliding movement of actin filaments was almost inhibited.

In the presence of 0.01–0.03 M guanidine-HCl, the degree of the decrease in ATPase activation seemed more enhanced compared to the decrease in the sliding velocity. However, at 0.04 M guanidine-HCl, 22% of the ATPase activation, while not accompanied by sliding movement, was still similar to that observed with urea.

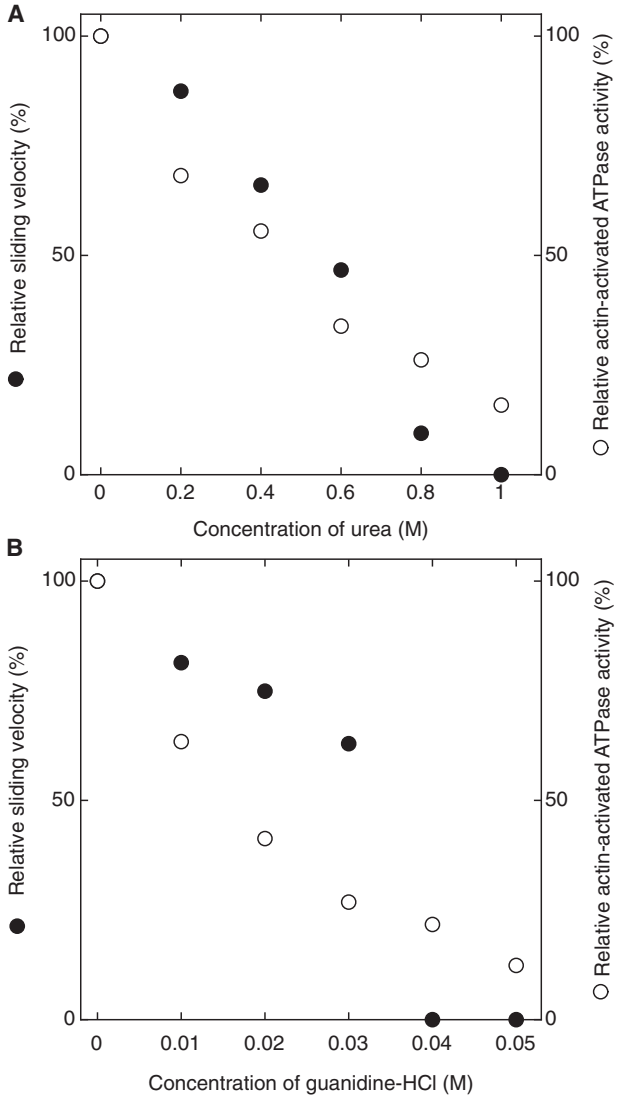

Fig. 8 Comparison between sliding velocities (filled circles) and actin-activated ATPase activities (open circles) in the presence of urea (A) and in the presence of guanidine-HCl (B). Sliding velocities for each condition were normalized by the original velocity in the absence of solutes (see data in Fig. 4). Actin-activated ATPase activity was the differences between ATPase activity with actin and that without actin (see data in Fig. 6). Actin-activated ATPase activity was also normalized to the original activity in the absence of solutes.

Discussion

Stability of actin filaments and HMM molecules

We first examined the possibility of denaturation of the actin filaments and HMM molecules in the presence of a urea solute.

Phalloidin-bound actin filaments were stable in the presence of urea up to 2.5 M. No difference in the length distributions of actin filaments in the presence and in the absence of urea was observed for the first 60 min (Fig. 3). In addition, both the changes in the fluorescence intensity of *bis*-ANS and in the spectral shift of intrinsic fluorescence were insignificant at urea concentrations below 2.0 M (Figs 1A and 2A). These facts also supported the idea that the ternary structure of actin constituents in filaments may be stable over the concentration of urea examined. With respect to the monomeric state of actin, Kuznetsova *et al.* (*17*) also reported that at urea concentrations ranging from 2.0 to 4.0 M, actin molecules are transformed into an intermediate, unfolded state and that complete unfolding occurs above 4.0 M.

Ortiz-Costa *et al.* (*18*) already reported that for myosin subfragment-1 (myosin S1), the exposure of the hydrophobic region to a solution environment is dependent on urea concentrations in the range of 0–2.0 M. Their spectral data shows that the intrinsic fluorescence intensity of myosin S1 markedly decreases with increases in urea concentration. However, a urea-dependent decrease in the intrinsic fluorescence intensity of HMM molecules was not observed in our present work (Fig. 1C). The reason for the inconsistency between these results is unknown. The measurements of *bis*-ANS-fluorescence revealed that the fluorescence intensity of *bis*-ANS in the presence of HMM molecules increased up to 1.5-fold in the presence of 1.0 M urea, which is in agreement with the results presented by Ortiz-Costa *et al.* (Fig. 2B). Although some changes in the ternary structure of HMM molecules were observed at 1.0 M urea, their ATPase activity in the absence of actin filaments remained unaffected.

Our motility assay revealed that the sliding movement recovered after the removal of the urea solute. In addition, the actin-activated ATPase activity recovered after dilution of the urea concentration. Hence, even in the presence of solutes that may completely inhibit the sliding movement of actin filaments, actin and HMM molecules did not presumably undergo irreversible damage to their conformation.

Difference of urea and guanidine-HCl

Each urea and guanidine molecule has a similar structure except that the central bond group is a carbonyl for urea and an imine for guanidine. Because of the intrinsic nature of the central bond group, guanidine-HCl is an ionic compound, which facilitates the denaturation of proteins by directly interacting with charged side chains (*12*, *19*). Guanidine-HCl can influence protein structures to a greater extent than urea (*20*). In fact, we observed that guanidine-HCl in the range of 0–1.0 M induces a 6.5-fold increase in the fluorescence intensity of *bis*-ANS with HMM, whereas urea in the range of 0–4.0 M induces only a 4.5-fold increase (Fig. 2B). Moreover, we found that guanidine-HCl was more effective in decreasing the sliding velocity of actin filaments and actin-activated ATPase activity compared with urea. Under conditions that completely suppress the sliding movement of the filaments, the change in the fluorescence intensity of *bis*-ANS bound to HMM at 0.05 M guanidine-HCl was smaller than that at 1.0 M urea (Fig. 2B). This result suggested that the denaturation caused by guanidine-HCl is sufficient to suppress acto-HMM interactions. Thus, the present results did not preclude the possibility that the urea-induced suppression is primarily caused by partial denaturation, rather than the direct effect of perturbed hydrogen bonds.

Effect of urea on the microenvironment around actomyosins

At low urea concentrations (<1.0 M), it seems unlikely that the solute may induce irreversible damage to the conformation of HMM and actin. Nonetheless, our experimental results demonstrated that the interaction between actin and HMM molecules was extremely influenced by the addition of urea molecules to the solution.

Because the urea solute acts not only as denaturant but also as an osmolyte, the viscosity of the solution increases with the addition of urea. Kawahara and Tanford (*21*) have already reported that the viscosity of the aqueous solution can be further elevated as much as 5% in the presence of 1 M urea. Because of the small increase in viscosity, it would be unlikely that the addition of urea may completely suppress the sliding movement of actin filaments on HMM molecules.

Although we do not have direct evidence of how the hydration of actomyosin is influenced by the addition of urea, some related works have reported that urea solutes affect the hydration layer of proteins more than the network of bulk water molecules (*6*, *8*, *9*). In fact, it is likely that urea molecules bind to the surface of proteins, while excluding the surrounding water molecules. This must be the place where selective solvation takes place. Auton *et al.* (*22*) actually suggested that when model peptides (*N*-acetyl triglycinamide and *N*-acetyl tetraglycineamide) are transferred into 1.0 M urea solution, a decrease of 40 ml/mol of hydration and an increase of 30 ml/mol of solvation of urea per peptide unit are observed. Accordingly, it is possible that the destabilization of protein structure is promoted when urea affects the hydration layer of proteins, and selective solvation subsequently occurs. In practice, we observed that a structural change in HMM molecules was partly induced by the presence of urea even at low concentrations in which complete unfolding do not take place.

Regarding the effects of hydration, Highsmith *et al.* (*1*) observed hydration changes in actomyosin during ATP hydrolysis by using an osmotic pressure technique. Their study revealed that dehydration of actomyosin occurs during ATP hydrolysis, while no such change is detected during the ATPase reaction of myosin not accompanied by actin molecules. This feature is compatible with our finding that urea influences acto-HMM activity, but not HMM ATPase activity. Nonetheless, urea was found to decrease acto-HMM ATPase activity, as opposed to enhancing the activity with increases in osmotic stress. When urea molecules exclude hydration waters from the actomyosin surface, the net effect of dehydration during the ATPase reaction may be decreased.

Similar co-solvent effects have also been investigated so far. In particular, Komatsu *et al.* (*23*) observed that hexanol, which can perturb the functional structure of the actomyosin complex, enhances myosin ATPase activity, while reducing the sliding velocity of actin filaments, because of the modulation of hydrophobic contributions to the internal structure of the ATP-myosin complex.

Shorter alkyl chain alcohols, such as ethanol, influence both the sliding velocity and ATPase activity in a mutually related manner (*24*). The strengths of both the velocity and ATPase activity were decreased to roughly 25% with increases in ethanol concentrations up to 1.0 M. This decrease depended on the concentration of KCl, indicating the significance of balance between electrostatic and hydrophobic interactions on the part of the actomyosin complex.

Both the ATPase activation and sliding movement are dependent on the affinity between actin and myosin molecules. Our present study of the actin concentration-dependence of ATPase activity revealed that actin filaments had reduced affinity for HMM molecules in the presence of 0.5 M urea. Furthermore, we also attempted co-sedimentation assays (data not shown) to estimate the amounts of HMM molecules bound to the actin filaments in the presence or absence of ATP. In the absence of ATP, the amounts of HMM bound to actin filaments slightly decreased to 97% of the levels at 1.0 M urea and further decreased to 90% at 0.05 M guanidine-HCl compared to the experiments with no such solutes. In the presence of ATP, on the other hand, the amounts of bound HMM were significantly decreased to 75% for urea and 35% for guanidine-HCl. This observation suggested that both urea and guanidine-HCl may weaken the binding affinity between actin filaments and myosin heads to some extent. Nonetheless, our experiments also showed that actin filaments bound to HMM molecules in the presence of urea below 1.0 M in the *in vitro* motility assays. The likely contribution of urea to binding may induce a weakly binding situation for the acto-HMM complex, impairing transmission of the mechanical force. A failure of unidirectional sliding movement may be a consequence of such an impairment. Despite that, actin filaments can dissipate the force by activating filamental fluctuations in the transversal direction (*25*).

In conclusion, the changes induced over the hydration water molecules around the actomyosin complex, including the weakening of the stability of their structures, may modulate the affinity between an actin filament and a myosin head for the transmission of force.

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Conflict of interest

None declared.

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