Molecular Motion

DOI: 10.1002/anie.200906139

Adhesion Effects of a Guanidinium Ion Appended Dendritic "Molecular Glue" on the ATP-Driven Sliding Motion of Actomyosin**

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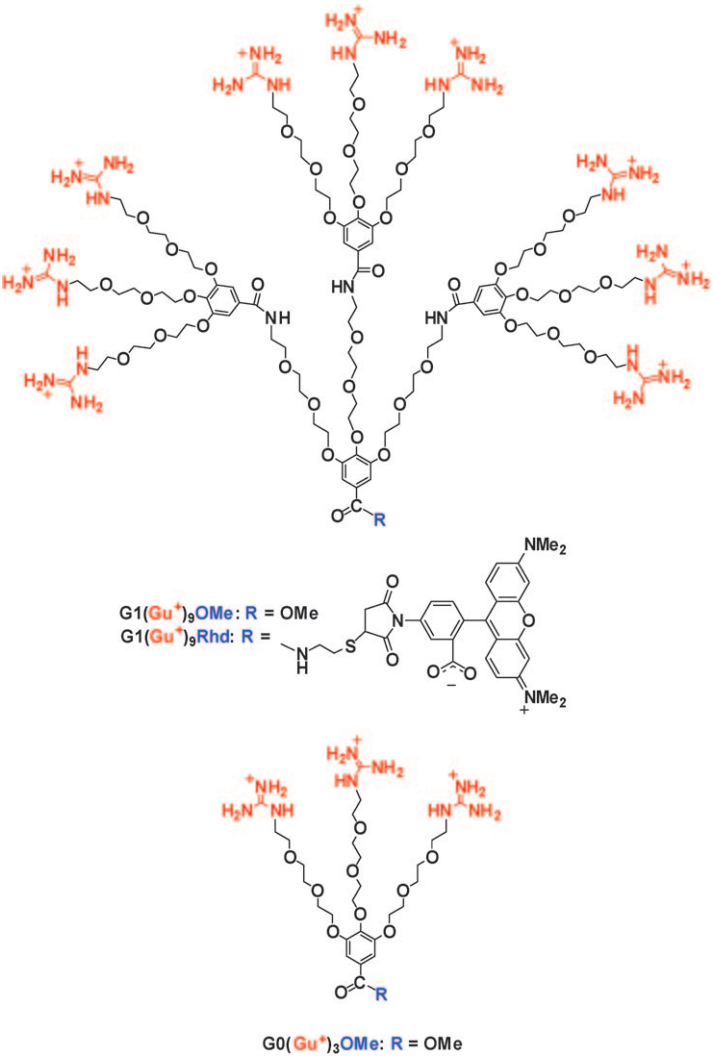
Biological systems sometimes utilize "adhesion" for performing required tasks. Typical examples include an amoeboid motion of white blood cells, which make use of carbohydrate moieties on their surface for the interaction with selectin. By using this adhesive interaction, selectin, which is produced at inflamed tissues, captures white blood cells from a rapid blood stream and allows them to operate site-selectively in wounded areas.[1] Herein we report a water-soluble dendrimer[2] that bears nine guanidinium ion pendants at its periphery (G1-$(Gu^+)_9$R; Scheme 1),[3] and serves as a synthetic inhibitor for the ATP-driven sliding motion of actomyosin by an adhesion mechanism (Figure 1). By using electrostatic and hydrogen-bonding interactions, the guanidinium ion can form a salt bridge with oxyanions,[4] which exist ubiquitously on the protein surface. By taking advantage of the dendritic architecture that allows for multivalent interactions,[5] G1$(Gu^+)_9$R strongly adheres to proteins even under buffered conditions.[3] In a previous report, we demonstrated that G1$(Gu^+)_9$OMe behaves like paclitaxel, which is capable of inhibiting the depolymerization of microtubules by tightly gluing their monomer units (tubulin dimers) together.[3] The successful stabilization of homotropic protein assemblies prompted us to investigate if G1$(Gu^+)_9$R can also stabilize heterotropic protein conjugates. We thought that the sliding motion of actomyosin, a heterotropic conjugate of actin and myosin, might be an interesting candidate for investigating the adhesive function of G1$(Gu^+)_9$R. The sliding motion of actomyosin is an important biological event, which is induced by ATP and is responsible for muscle contraction.[6] At the initial stage of this event, ATP is hydrolyzed by myosin ATPase, whereupon a mechanical motion of myosin is generated. This motion is translated into the sliding motion of actin filaments by taking advantage of the fact that actomyosin dynamically changes its association constant from approximately 10^4 to $10^7\,M^{-1}$ in response to the binding and hydrolysis, respectively, of ATP.[7] We therefore envisaged that the sliding motion of actomyosin might be arrested if G1$(Gu^+)_9$R can suppress the dissociation of actomyosin into actin and myosin by an adhesive interaction.



Scheme 1. Structures of dendritic G1$(Gu^+)_9$R and G0$(Gu^+)_3$R bearing pendant guanidinium ions (chloride salts).

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[**] This work was partially supported by the Ministry of Education, Science, Sports, and Culture (Japan). K.K. acknowledges a Grant-in-Aid for Young Scientists (S) and a grant from the Toray Science Foundation. ATP = adenosine triphosphate.

Supporting information (materials and experimental procedures) for this article is available on the WWW under http://dx.doi.org/10.1002/anie.200906139.

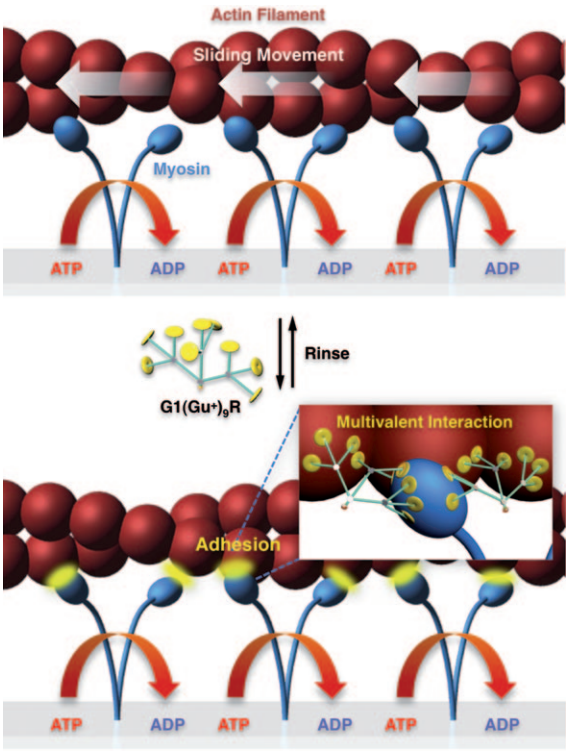

Figure 1. Illustration of a possible mechanism of the inhibition effect of dendritic molecular glue G1$(Gu^+)_9$R on the ATP-driven sliding motion of actomyosin. G1$(Gu^+)_9$R adheres to the heterotropic interface of actin and myosin and restricts the sliding motion of actin filaments on a myosin-functionalized coverslip. Actin filaments can retrieve their original motility by the removal of G1$(Gu^+)_9$R.

Isothermal titration calorimetry (ITC),[8] which was carried out by stepwise addition of G1$(Gu^+)_9$OMe (Scheme 1) to myosin at 25 °C in 20 mM HEPES buffer (0.6 M KCl, pH 7.0; HEPES = 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), displayed an exothermic profile with multistep changes in peak intensity upon increasing the amount of G1$(Gu^+)_9$OMe (Figure 2a,b). This ITC feature indicates that multiple G1$(Gu^+)_9$OMe molecules are bound in a stepwise manner to myosin. Although the ITC measurements were carried out in buffered solutions, the association constants K_1–K_4, as evaluated from the observed ΔH_{ITC} values, are very high; $K_1 = 8.3\times10^7$, $K_2 = 4.6\times10^7$, $K_3 = 1.6\times10^6$, and $K_4 = 7.1\times10^5\,M^{-1}$ (Figure 2b). Since an actin filament is a dynamic assembly, ITC was not applicable to the evaluation of its interaction with G1$(Gu^+)_9$OMe. However, upon mixing with rhodamine-appended G1$(Gu^+)_9$Rhd[3] (Scheme 1), the actin filaments showed a red fluorescence and could be successfully visualized by confocal laser scanning microscopy (Figure 3a; $\lambda_{ex} = 543$ nm). Therefore, G1$(Gu^+)_9$R can strongly adhere to actin filaments as well as myosin (Figure 3b).

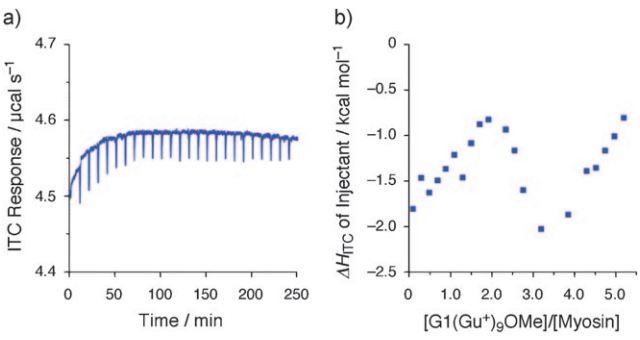

Figure 2. Isothermal titration calorimetry (ITC) profiles in KCl (0.6 M) containing HEPES buffer (20 mM, pH 7.0). a) Titration of myosin (3.5 μM) with G1$(Gu^+)_9$OMe (100 μM) in a range of [G1$(Gu^+)_9$OMe]/[myosin] from 0 to 5.2. b) ΔH_{ITC} plots calculated from the thermograms of G1$(Gu^+)_9$OMe/myosin ($K_1 = 8.3\times10^7$, $K_2 = 4.6\times10^7$, $K_3 = 1.6\times10^6$, and $K_4 = 7.1\times10^5\,M^{-1}$).

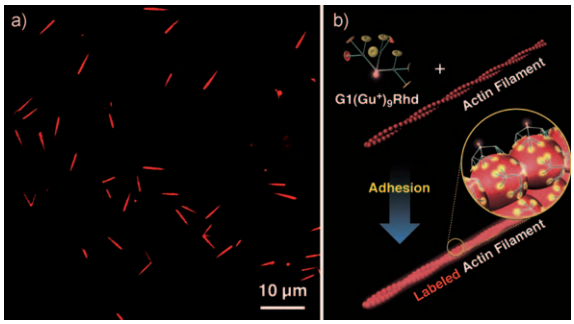

Figure 3. a) Confocal laser scanning micrograph of actin filaments in the presence of G1$(Gu^+)_9$Rhd (100 μM), upon excitation at 543 nm in a solution of KCl (100 mM) in HEPES buffer (20 mM, pH 7.0) with glucose (4.5 mg mL^{-1}), catalase (0.036 mg mL^{-1}), glucose oxidase (0.216 mg mL^{-1}), and 2-mercaptoethanol (0.5%). b) Illustration of a fluorescently labeled actin filament upon interaction with rhodamine-appended G1$(Gu^+)_9$Rhd.

As reported previously,[9] when actin filaments fluorescently labeled with phalloidin-tetramethylrhodamine were employed, their ATP-driven sliding motions on a myosin-functionalized coverslip were visualized by fluorescence microscopy upon excitation at 550 nm.[10] For investigating the effects of G1$(Gu^+)_9$OMe on the sliding motion of actin filaments, we utilized a flow-cell setup fabricated as follows: A glass coverslip was washed with an aqueous solution of KOH (0.1M) and deionized water (3 times), and then placed on a slide glass with two strips of a double-sided tape as spacers (Figure S1 in the Supporting Information). Then, the flow cell was covered with a solution of myosin (30 μL, 40 mg mL^{-1}) in HEPES buffer (20 mM, pH 7.0) containing KCl (25 mM) and $MgCl_2$ (5 mM) and incubated for 60 s. After unbound myosin was rinsed out by injecting myosin-free HEPES buffer (45 μL), the flow cell was filled with a solution of bovine serum albumin (BSA; 0.5 mg mL^{-1}) in HEPES buffer (20 μL) and incubated for 60 s to inactivate the nonfunctionalized parts of the coverslip surface. Then, unbound BSA was rinsed out with BSA-free HEPES buffer (45 μL). Finally, the flow cell was filled at 26 °C with an ATP-buffer solution (20 μL) of fluorescently labeled actin filaments (0.8 ng).[9] As observed by fluorescence microscopy of the resulting flow cell (Figure 4a,b), actin filaments (0.8 ng) indeed underwent a sliding motion on the myosin-functionalized coverslip with an average velocity of 4.6 μm s^{-1}, which is consistent with the reported value.[9]

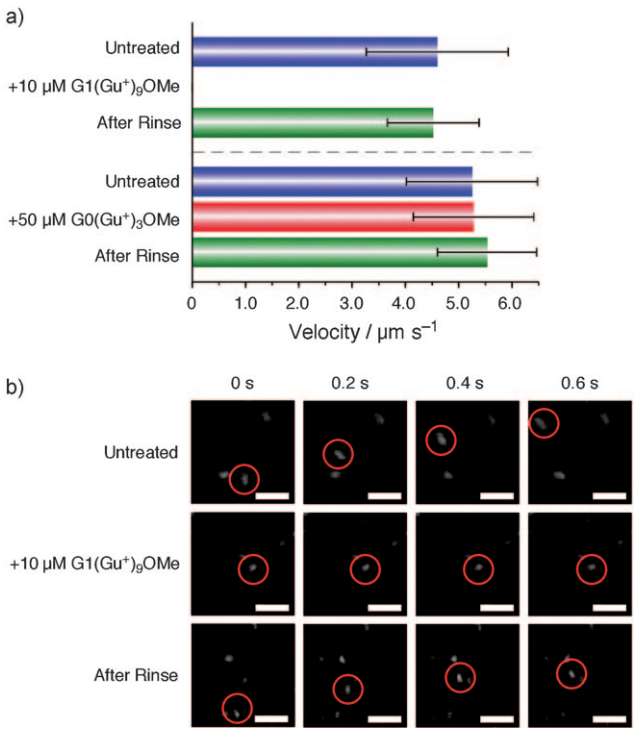

Figure 4. a) Average velocities of the motions of arbitrarily selected 30 actin filaments on a myosin-coated coverslip at 26 °C in ATP buffer (HEPES (20 mM; pH 7.0) containing KCl (25 mM), $MgCl_2$ (5 mM), ATP (2 mM), glucose (4.5 mg mL^{-1}), catalase (0.036 mg mL^{-1}), glucose oxidase (0.216 mg mL^{-1}), and 2-mercaptoethanol (0.5 %)), before (blue) and after (red) the injection of an ATP buffer solution of $G1(Gu^+)_9OMe$ (10 μM) or $G0(Gu^+)_3OMe$ (50 μM), followed by rinsing with dendrimer-free ATP buffer (green). b) Fluorescence microscopic traces of the motions of actin filaments upon excitation at 550 nm (0–0.6 s) under the three different conditions described above. Scale bars = 3 μm.

When an ATP-buffer solution of $G1(Gu^+)_9OMe$ (20 μL, 10 μM) was injected into the flow cell, the actin filaments, as expected, became completely immobile (Figure 4a, b). On the other hand, when the interior of the flow cell was rinsed with an excess of dendrimer-free ATP-buffer (45 μL), the actin filaments, arrested by the action of $G1(Gu^+)_9OMe$, started to move again. We confirmed that myosin still preserves its ATPase activity after treatment with $G1(Gu^+)_9OMe$. Thus, $G1(Gu^+)_9OMe$ was dissolved in KCl buffer, and the resulting mixture ($[G1(Gu^+)_9OMe] = 10$ μM) was submitted to an activity assay with the BIOMOL Green kit. At 26 °C, the ATPase activity of myosin was evaluated as 61.3 ± 1.1 nmol mg^{-1} min^{-1}, which is very similar to that observed in the absence of $G1(Gu^+)_9OMe$ (57.3 ± 0.7 nmol mg^{-1} min^{-1}). The ATPase activity thus observed for $G1(Gu^+)_9OMe$-treated myosin suggests that the dendritic "molecular glue" does not hamper the ability of myosin to move in response to ATP. Therefore, the arrested motion of actin filaments in the presence of $G1(Gu^+)_9OMe$ (Figure 4) can be accounted for by the suppression of actomyosin to dissociate into actin and myosin because of their tight heterotropic adhesion with $G1(Gu^+)_9OMe$. It is noteworthy that when actin filaments and the myosin-coated coverslip, which were both pretreated with $G1(Gu^+)_9OMe$ (10 μM), came into contact, the actin filaments formed only floating agglomerates, which were washed out from the flow cell upon injection of ATP-buffer. This result shows that the motor proteins, which were once covered entirely by $G1(Gu^+)_9OMe$, were no longer adhered to one another. In contrast to $G1(Gu^+)_9OMe$, the lower-generation $G0(Gu^+)_3OMe$,[3] which bears only three guanidinium ion pendants (Scheme 1), did not affect the motion of actin filaments, even when a large amount of $G0(Gu^+)_3OMe$ (50 μM) was injected into the flow cell (Figure 4a). Thus, the multivalency of $G1(Gu^+)_9OMe$ plays a crucial role in its large adhesiveness toward the motor proteins.

Heavy meromyosin (HMM) is a simplified version of myosin, and consists only of its essential component required for the sliding motion.[11] As reported previously, in the presence of ATP, actin filaments moved on the HMM-functionalized coverslip. The coverslip modified with HMM is advantageous since the motion of actin filaments on its surface can be followed more accurately than on the myosin-functionalized version. By virtue of this accuracy, we found that the moving velocity of actin filaments can be modulated by the amount of $G1(Gu^+)_9OMe$ employed. As shown in Figure 5, the average velocity was smaller as $[G1(Gu^+)_9OMe]$

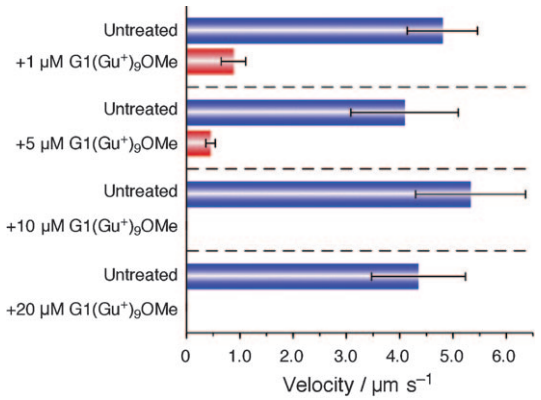

Figure 5. Average velocities of the motions of arbitrarily selected 30 actin filaments on a heavy meromyosin (HMM)-coated coverslip at 26 °C in ATP buffer, before (blue) and after (red) the injection of ATP buffer solutions of $G1(Gu^+)_9OMe$ (1, 5, 10, and 20 μM).

(the concentration of $G1(Gu^+)_9OMe$) was higher. For example, when $[G1(Gu^+)_9OMe]$ was lower than 5 μM, actin filaments in the presence of ATP still moved on the HMM-functionalized coverslip. However, when $[G1(Gu^+)_9OMe]$ was equal to or greater than 10 μM, the actin filaments became totally immobile. The decelerated motion of actin filaments, which is dependent on $[G1(Gu^+)_9OMe]$, suggests an interesting possibility that the adhesive interaction, unless sufficiently strong, is temporarily broken by a mechanical force given by the ATP-driven motion of myosin, but can be regenerated at a heterotropic protein interface that is newly formed upon sliding motion. This phenomenon represents a kinetic aspect of the protein adhesion with $G1(Gu^+)_9R$.

In conclusion, we have demonstrated that the dendritic molecular glue G1$(Gu^+)_9$R that bears multiple guanidinium ion pendants at its periphery strongly adheres to motor proteins such as actin and myosin even under buffered conditions, where the binding constant is as large as $10^8\,M^{-1}$. The strong adhesion capability means that G1$(Gu^+)_9$R stabilizes the heterotropic conjugate of actin and myosin (actomyosin), and decelerates or totally arrests its ATP-driven sliding motion. By varying the amount of G1$(Gu^+)_9$R, the moving velocity of actomyosin can be changed. In this example, G1$(Gu^+)_9$R appears to operate differently from a biological inhibitor such as troponin/tropomyosin,[12] which blocks the binding sites of the motor proteins to intercept their conjugation. Incorporation of stimuli-responsive functionalities into the dendritic molecular glue is one of the interesting subjects that is worthy of further investigation.

Received: October 31, 2009
Published online: March 12, 2010

Keywords: adhesion · dendrimers · molecular motion · multivalent interactions · proteins

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