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Delayed dissociation of in vitro moving actin filaments from heavy meromyosin induced by low concentrations of Triton X-100

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Abstract

The in vitro motility of fluorescent actin filaments over heavy meromyosin (HMM) was studied in the presence of the nonionic detergent Triton X-100. Below 0.004% Triton X-100 concentration, motility was not affected. Above 0.007%, motility was not observed because actin filaments were dissociated from HMM. In the Triton X-100 concentration range of 0.004–0.007%, the sliding actin filaments dissociated from HMM with a delay. The dissociation delay time decreased with increasing Triton X-100 concentration, increasing ATP (adenosine–5′–triphosphate) concentration, and increasing temperature. The delayed acto-HMM dissociation was absent when weak-binding kinetic intermediates of the myosin ATPase cycle (M.ATP and M.ADP–P_i) were used. The presence of sliding movement was necessary to evoke the delayed acto-HMM dissociation. The acto-HMM dissociation delay was independent of actin filament length. For a given Triton X-100 concentration, the dissociation delay time was found to be inversely proportional to sliding velocity, indicating that actin filaments travel a more or less constant distance prior to dissociation from HMM. The actin-activated HMM ATPase activity was not inhibited by Triton X-100; rather, it was slightly enhanced. The results imply the presence of a motility-associated conformational change in acto-HMM. © 1997 Elsevier Science B.V.

Keywords: Nonionic detergent; Muscle contraction; In vitro motility; ATPase; Chemomechanical transduction

1. Introduction

Muscle contraction and various forms of cell motility occur as a result of the interaction between actin and myosin. Actin binding to myosin accelerates the rate of Mg–ATP hydrolysis by myosin in solution by one or two orders of magnitude [1]. The activation of myosin by actin is thought to be a manifestation of the mechanism of energy transduction by which actomyosin uses energy from Mg–ATP binding and hydrolysis to produce force and shortening. Through investigating the activation of myosin, by actin or other molecules, one hopes to gain insight into the energy transduction mechanism.

Organic molecules have previously been used to

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activate or modify the ATPase activity of myosin. Small aliphatic alcohols and ethers were found to increase the rate of myosin or subfragment-1 (S-1) activity [2–8]. In a recent study, ethanol was shown to alter the in vitro motility of actin filaments over myosin and myosin ATPase in an ionic strength-dependent manner [9]. Nonionic detergents have been widely used in studying the activation of myosin [1,10,11]. A reversible modification of the myosin ATPase activity and actin affinity was found upon the effect of nonionic detergents [1,10,11]. The reversible inhibition of myosin K–ATPase activity caused by the Triton X series was interpreted as evidence that the bound detergent created a barrier to ATP binding [10]. The reversible enhancement of S-1 Mg–ATPase activity by $C_{12}E_9$ (C_XE_Y, *n*-alkyl polyoxyethylene ether with X carbons in the alkyl chain and Y ethylene glycol units in the polyoxyethylene chain) was interpreted as evidence suggesting that the alkyl moieties of the detergent penetrated into the interior of S-1 and induced a conformational change that modified its function [1]. A further study using $C_{12}E_8$ and CHAPS pointed to that not detergent micelles but myosin-bound detergent molecules are responsible for modifying the myosin ATPase [11]. Although nonionic detergents have been shown to alter the myosin ATPase and reduce acto–myosin affinity, their effect on the interaction of actin and myosin in the in vitro motility assay is not known.

The aim of the present study was to examine the effect of the nonionic detergent Triton X-100 on the interaction between F-actin and HMM in the in vitro motility assay. The assay consists only of the components necessary for contraction (F-actin and HMM); therefore, the direct effects of Triton X-100 on the contractile apparatus can be examined. In the concentration range of 0.004–0.007%, Triton X-100 caused a delayed, rapid and complete dissociation of sliding actin filaments from HMM. To explore the mechanisms of Triton X-100 action, in vitro motility and binding assays were carried out under a variety of conditions, including the use of weak-binding kinetic intermediates of the myosin ATPase cycle. The results revealed that the occurrence of sliding movement was necessary in evoking the delayed dissociation, implying that Triton X-100 interferes with a motility-associated conformational change in acto-HMM.

2. Materials and methods

2.1. Preparation of proteins

Myosin was prepared by steps described previously [12]. HMM was prepared fresh from myosin stored in 50% glycerol at −20°C followed by the removal of irreversible rigor heads [13]. Vanadate-trapped heavy meromyosin was prepared as previously described [14]. Actin was prepared from rabbit back muscle by described methods [15]. F-actin was fluorescently labelled with molar excess of tetramethyl-rhodamine-isothiocyanate-phalloidin (TRITC–Ph, Sigma Chemical Company, St. Louis, MO). Purity of the protein preparations was checked using SDS–polyacrylamide electrophoresis on a 10% gel [16]. The Sigma SDS-6H kit was used as molecular-weight standard.

2.2. In vitro motility assay

In vitro motility assay experiments were carried out according to previously described methods [13] with modifications. Fluorescent actin filaments were visualized in a Zeiss Universal upright epifluorescence microscope equipped with a mercury (HBO200) arc lamp, Planapo 63 × /1.4 NA oil immersion objective (Zeiss, Germany), and rhodamine interference filter set (Omega Optical, Brattleboro, VT). Filament images were detected by a microchannel plate (MWK Industries, Corona, CA) optically coupled to a CCD Camera (DXC-102P, Sony, Japan), and recorded on high-bandwidth 8 mm (Hi8) video tape (CCD V-5000E, Sony, Japan). Temperature in the in vitro assay was adjusted by circulating water (from a thermostat) in a coil wrapped around the objective. Experiments were carried out at 30°C, except where otherwise indicated. The flow-through microchamber used in the motility assay was similar to that described previously [12], adapted to the upright microscope system. The protein samples and buffer solutions were washed through the microchamber in the following general order. First, HMM (diluted in Assay Buffer (AB, 25 mM imidazole–HCl, pH 7.4, 25 mM KCl, 4 mM $MgCl_2$, 1 mM EGTA)) was pipetted in the microchamber, at a concentration of 50 μg/ml, and allowed to bind to the nitrocellulose-coated surface. The 50 μg/ml

concentration of HMM has previously been shown to saturate the nitrocellulose surface [17]. Unbound HMM was then washed out with AB solution. BSA (bovine serum albumin, usually used to block non-specific binding sites) was omitted to avoid possible artificial effects by binding the detergent [18]. Control and previous experiments [12] showed, however, that omitting BSA did not affect characteristics of actin filament sliding. Fluorescent actin filaments were added at a concentration of 70 ng/ml and allowed to bind to HMM for one minute. Unbound actin was washed out by AB buffer with 10 mM β-mercaptoethanol and an oxygen–scavenger enzyme system added to reduce photobleaching [19]. Motility of actin filaments was initiated by adding ATP at a concentration of 1 mM. Low ionic strength (42 mM) was maintained throughout the experiments to avoid artificial actin filament dissociation known to occur at high ionic strengths [20,21]. In some experiments, 1% (w/v) methylcellulose was added to raise the macroscopic viscosity of the solution (from ~ 1 to ~ 2000 centipoise) and inhibit the lateral diffusion of actin filaments [17]. In these experiments the saturating density of HMM was maintained.

2.3. Measurement of acto-HMM dissociation delay

The acto-HMM dissociation delay time was measured as the time passed from the addition of ATP in the sample chamber to the 50% reduction of actin filament sliding velocity due to the dissociation of the filaments from the HMM-coated surface. While the latter was well defined, the addition of ATP in the sample chamber caused uncertainty of determining the actual starting point of the delay, and hence the delay time. ATP was added in the sample chamber by adding solution at one end of the chamber and wicking the solution through the chamber by using filter paper. The solution exchange typically took about ten seconds, but depended strongly on the characteristics of the sample chamber, viscosity of the solution, and the type of the filter paper used. The variation in the rate of complete solution exchange resulted in a variable delay-time offset from one series of experiments to the other. Within one series of experiments, however, the delay time and particularly the tendency of its change upon varying the Triton X-100 or ATP concentrations was highly consistent. The dissociation delay times in the figures indicate the time measured from the end of the solution exchange to the dissociation, and disregard the time required for the solution exchange.

2.4. In vitro binding assay

Fluorescent actin filaments were allowed to bind to HMM immobilized on a nitrocellulose-coated glass coverslip, similarly to the arrangement in the in vitro motility assay. The amount of actin bound to HMM was measured by counting the number of actin filaments per microscopic field of view, and measuring their length. The amount of actin was then expressed as either the number or the total length of actin filaments per microscopic field of view. The in vitro binding assays were carried out under a variety of conditions and Triton X-100 concentrations. The investigated Triton X-100 concentrations included 0.005%, where delayed acto-HMM dissociation is observed and a high concentration (usually 0.1%) where prompt acto-HMM dissociation is observed. While this in vitro binding assay does not provide K_m values in contrast to the classical free-solution equilibrium binding experiments, changes in the strength of actin binding to HMM can be monitored [22].

2.5. Actin-activated HMM ATPase assay

The ATPase rate was measured according to the method of Ohno and Kodama [23] with modifications. HMM (0.75 μM) was mixed with F-actin (0.5 μM) in AB buffer and incubated at 30°C for two minutes. Triton X-100 (in the range of 0.001–0.1%) and ATP (1 mM) were rapidly added, and the reaction was incubated at 30°C. After 10 s, 2, 4 and 6 min, 0.27 ml of the reaction mixture was withdrawn and added to 0.2 ml ice-cold, 0.67 M perchloric acid (PCA). Following a 90-min incubation on ice, the samples were centrifuged at 1 500 g, 4°C, for two minutes. 0.2 ml of the supernatant was added to 2 ml Malachite Green reagent (50 mM Na–molybdate, 0.8 mM Malachite Green in 1 M HCl, freshly mixed with an equal volume of 0.3 M PCA). After adding 0.2 ml 34% Na–citrate, the samples were incubated at 25°C for 12 min. Optical density was measured at

650 nm. Results were expressed as μmol of phosphate released per mg of HMM. For calibration, a phosphate concentration series was generated from a 1 mM KH_2PO_4 stock solution.

2.6. Data processing and statistics

The images of actin filaments were analyzed after digitalization by using an Apple Macintosh IIsi computer with an LG-3 frame grabber board (Scion, Frederick, MD), running public domain image analysis software (Image v.1.55, Wayne Rasband, NIH, Bethesda, MD). Filament velocities (instantaneous and running average) were determined in 8 to 80-s-long sequences of images with a time resolution of 0.1 s. Filament lengths were measured by manually tracking their contour using the freehand tool of the image analysis software. For spatial calibration, a 10 μm optical grating (Zeiss, Germany) was used. Data are presented as mean with standard deviation or standard error of the mean, as indicated.

2.7. Chemicals and solutions

All chemicals were reagent grade. ATP (special quality, Boehringer Mannheim, Indianapolis, IN) was used by dilution from a 300 mM stock solution prepared as previously described [12]. Methylcellulose (Sigma No. M-0512) was added from a 2% stock solution in AB solution with 0.01% NaN_3 added. Triton X-100 (BDH Chemicals, Poole) concentration was adjusted by dilution from a freshly prepared 1% aqueous solution. Catalase and glucose oxidase were from Boehringer Mannheim (Indianapolis, IN). All other chemicals were from Sigma (St. Louis, MO).

3. Results

3.1. Effect of Triton X-100 on in vitro acto-HMM motility

Below 0.004% Triton X-100 concentration and at an ATP concentration of 1 mM, the in vitro motility of actin filaments over HMM was not affected. Above 0.007% Triton X-100 concentration and at an ATP concentration of 1 mM, actin filaments were completely dissociated from HMM, and motility could not be observed. In the concentration range of 0.004–0.007%, however, Triton X-100 caused a dissociation of actin filaments from the HMM-coated surface that occurred with a delay. Fig. 1 shows the diagrammatic movement of a representative actin filament following the addition of 1 mM ATP and 0.005% Triton X-100. Following the addition of 1 mM ATP and 0.005% Triton X-100, actin filaments began sliding over the HMM-coated glass coverslip with a velocity of 6.86 μm/s ($\pm$0.12, SEM). After about 4 s, the filaments suddenly dissociated from the surface, and floated into the solution. The dissociation occurred rapidly; it took place within one second. Essentially all filaments dissociated from the surface, with only a few immobile filaments remaining focally attached. Fig. 2a shows the velocity of actin filaments as a function of time in the presence of 1 mM ATP and 0.005% Triton X-100. About four seconds after the addition of ATP, sliding velocity dropped sharply, due to the dissociation of the filaments. Upon increasing the Triton X-100 concentration, the dissociation delay time decreased. At 0.006% Triton X-100 concentration (Fig. 2b), actin filaments dissociated from HMM about two seconds after the addition of ATP and the detergent. Upon

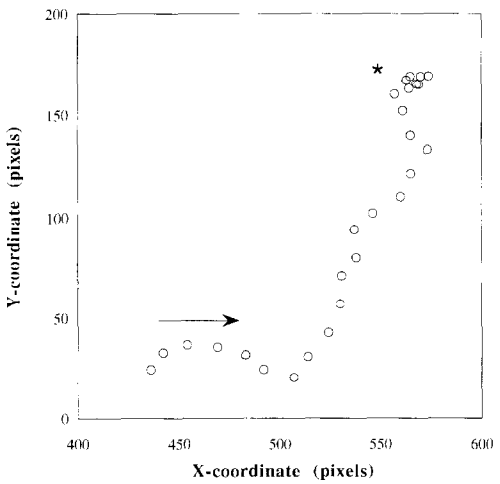

Fig. 1. Diagrammatic representation of an actin filament sliding on an HMM-coated surface, in the presence of 1 mM ATP and 0.005% Triton X-100. *X* and *Y* axes are spatial coordinates in pixels. Arrow indicates direction of movement. Time lapse between points is 0.2 s. At the location indicated by asterisk (*), the filament dissociated into solution.

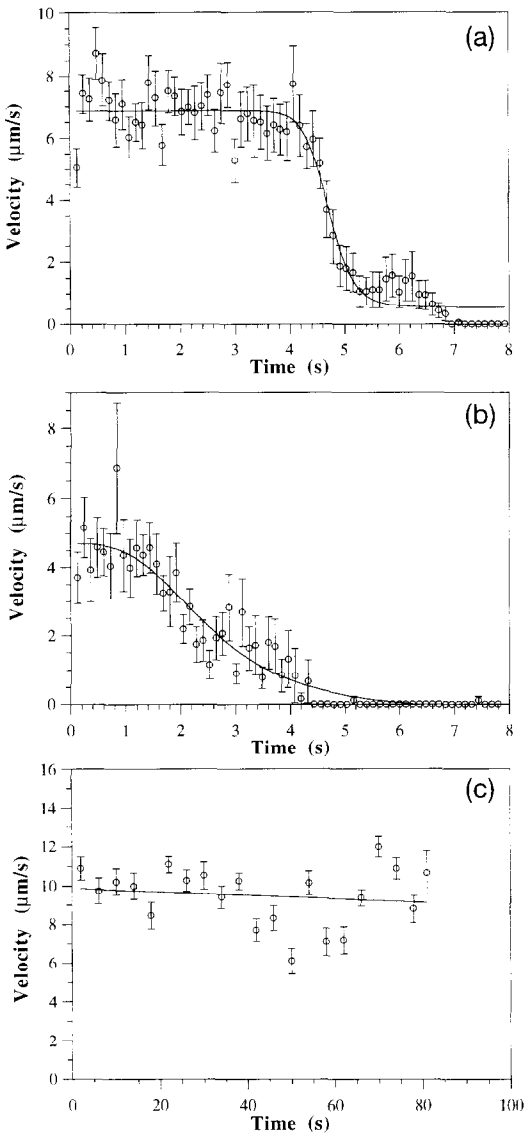

Fig. 2. Effect of Triton X-100 on actin filament velocity. (a) Actin filament velocity as a function of time, in the presence of 1 mM ATP and 0.005% Triton X-100. Time 0 is at the completion of the addition of the ATP and Triton X-100-containing solution in the sample chamber. Data were fit with the logistic function $f(x) = (a-d)/[1+(x/c)^b]+d$, where a and d are the maximal and minimal velocities, respectively, b is the slope parameter, and c is the time at the half-maximal value of filament velocity. (b) Actin filament velocity as a function of time, in the presence of 1 mM ATP and 0.006% Triton X-100. (c) Control experiment: actin filament velocity as a function of time, in the presence of 1 mM ATP and in the absence of Triton X-100. Error bars represent the standard error of the mean instantaneous velocity for all the filaments on the surface in the field of view, obtained in one series of experiments. The number of filaments was between 10 and 25.

increasing the Triton X-100 concentration further, the dissociation delay time shortened further, until, at 0.008% Triton X-100, actin filaments dissociated with no resolvable delay. In control experiments, in the absence of Triton X-100 and at 1 mM ATP concentration, actin filaments moved over HMM with a velocity of 10 μm/s. There was no significant decrease in actin filament velocity (Fig. 2c). Filaments stayed attached to the HMM-coated surface, and dissociation did not occur. Fig. 3 summarizes the results of the in vitro motility assays in the presence of Triton X-100 in the concentration range of 0.004–0.007%. The dissociation delay time is shown as a function of Triton X-100 concentration. The delay time decreased from 18 s in the case of 0.004% Triton X-100 to 2 s in the case of 0.007% detergent. At 0.008% Triton X-100, no delay could be resolved.

The addition of Triton X-100 (in the concentration range of 0.004–0.007%) caused not only a delayed acto-HMM dissociation, but also a reduction of maximal velocity (V_{max}) of sliding movement. Following the addition of Triton X-100 and 1 mM ATP, the sliding movement of actin filaments began with a velocity lower than that in the control experiments. The velocity stayed constant until the rapid dissociation of the filaments from HMM. Fig. 4 shows V_{max} before dissociation, as a function of Triton X-100 concentration. In the absence of Triton X-100, actin filaments moved with a velocity of 9.47

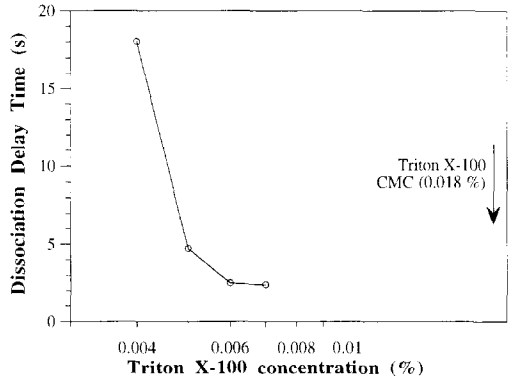

Fig. 3. Acto-HMM dissociation delay time as a function of Triton X-100 concentration. The critical micelle concentration (CMC) of Triton X-100 is indicated with the arrow. The data shown were extracted from the results displayed in Fig. 2.

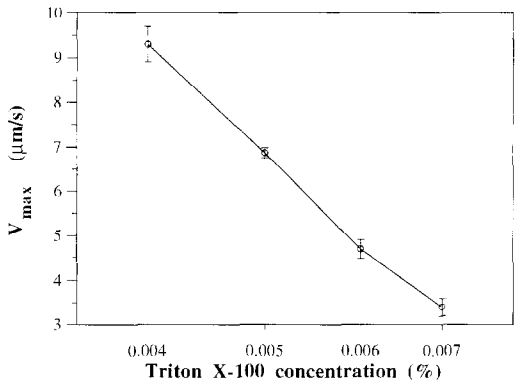

Fig. 4. Mean maximal sliding velocity (V_{max}) prior to dissociation as a function of Triton X-100 concentration. Error bars represent the standard error of the mean V_{max} for all the filaments in the field of view.

μm/s (±0.33, SEM). In the Triton X-100 concentration range of 0.004–0.007%, V_{max} decreased gradually with increasing detergent concentration, from 9.3 μm/s (±0.39, SEM) to 3.4 μm/s (±0.2, SEM).

To test whether the effect of Triton X-100 was reversible, the detergent, along with the dissociated actin filaments, was washed out of the flow cell, and new actin filaments were added. The newly added actin filaments bound to the HMM-coated surface. Upon the addition of 1 mM ATP, motility began with no dissociation. Thus, the effect of Triton X-100 was reversible.

Following dissociation from HMM upon the addition of Triton X-100, sliding movement of the actin filaments was abolished, and only their Brownian motion in solution could be observed. To test whether sliding movement could be preserved by inhibiting Brownian motion, 1% methylcellulose was added to the assay. Methylcellulose inhibits the lateral diffusion of actin filaments, but does not interfere with their sliding movement [17]. In the presence of 1% methylcellulose, sliding movement of actin filaments was preserved at Triton X-100 concentrations up to 0.05% (0.8 mM). The velocity of actin filaments progressively decreased with increasing Triton X-100 concentration beyond 0.05% (Fig. 5). There was no resolvable actin filament dissociation from the HMM-coated surface. Thus, by inhibiting the Brownian movement of actin filaments, their delayed dissociation from HMM induced by Triton X-100 (in the range of 0.004–0.007%) was prevented.

3.2. Effect of Triton X-100 on in vitro acto-HMM binding

To test whether the dissociation of F-actin from HMM upon the addition of Triton X-100 is the result of reduced actin affinity to HMM, in vitro binding assays were carried out. When Triton X-100 was added to actin filaments bound to HMM in rigor, no dissociation was observed (Fig. 6a). Even at high Triton X-100 concentrations (up to 1%), the filaments remained attached to the HMM-coated surface. There was no sign of dissociation. Thus, Triton X-100 did not cause acto-HMM dissociation once the two molecules were bound to each other in the rigor state. When the HMM surface was treated with Triton X-100 prior to the addition of F-actin, the amount of actin bound to HMM decreased. The total actin filament length per field of view gradually decreased as a function of Triton X-100 concentration used for pretreating HMM (Fig. 6b). However, even at a Triton X-100 concentration as high as 1%, there was no complete dissociation, as actin filaments bound to HMM.

To analyze whether the reduced acto-HMM affinity is associated with a weak-binding kinetic inter-

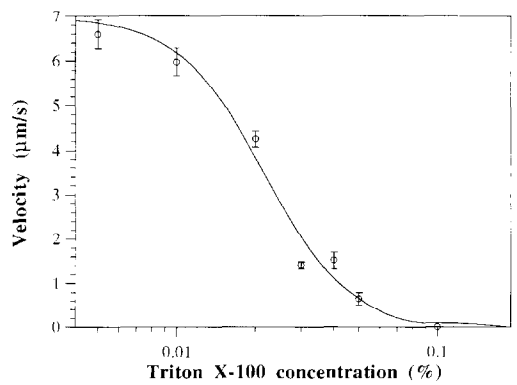

Fig. 5. Sliding velocity as a function of Triton X-100 concentration in the presence of 1% methylcellulose. Mean velocities ± SEM are shown. The data were fit with the logistic function $f(x) = (a - d)/[1 + (x/c)^b] + d$, where a and d are the maximal and minimal velocities, respectively, b is the slope parameter, and c is the Triton X-100 concentration at the half-maximal value of filament velocity.

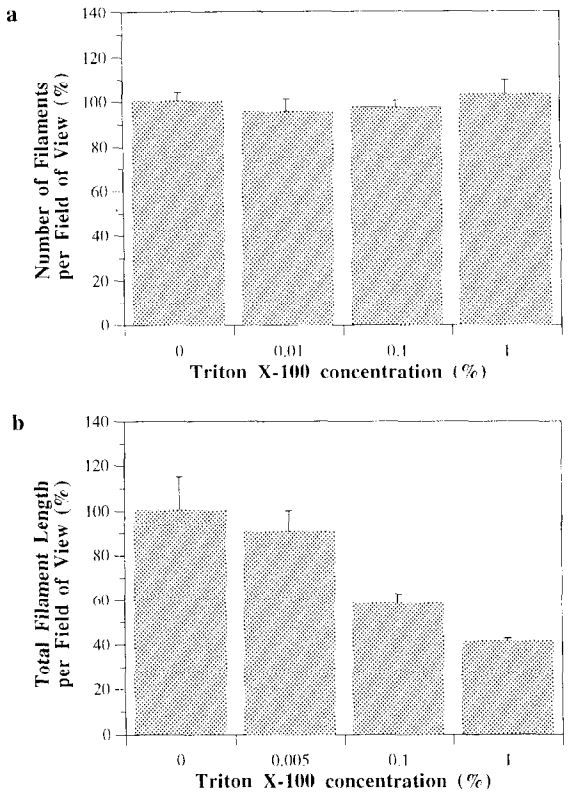

Fig. 6. Effect of Triton X-100 on in vitro acto-HMM binding. (a) Number of actin filaments (per microscopic field of view) attached to HMM-coated surface in rigor, as a function of Triton X-100 concentration. (b) Total actin filament length per field of view of actin filaments bound to HMM pretreated with Triton X-100, as a function of Triton X-100 concentration. Data are shown as mean ± SEM.

mediate of the myosin ATPase cycle, ATP-bound (M.ATP) and ADP–P_i-bound (M.ADP–P_i) states were experimentally modeled and used in the in vitro binding assay. The M.ATP state was modelled by using the nonhydrolyzable ATP analog ATP–γ-S (adenosine–5′–O–(3-thiotriphosphate)) [24–26]. First, the nitrocellulose-coated surface of the microchamber was coated with HMM. Fluorescent actin filaments were then allowed to bind to HMM. Subsequently, 1 mM ATP–γ-S was added in the microchamber together with Triton X-100. In the presence of 1 mM ATP–γ-S, increasing concentrations of Triton X-100 did not cause dissociation of F-actin from HMM (Fig. 7a). The M.ADP–P_i state of myosin was modelled by using vanadate-trapped HMM [27]. HMM, treated with 1 mM ADP–V_i, was used to coat the surface of microchamber. Fluorescent actin filaments were then added and allowed to bind to HMM for 1 min. Subsequently, Triton X-100 was added in the microchamber. Increasing concentrations of Triton X-100 failed to cause actin filament dissociation from vanadate-trapped HMM (Fig. 7b). Thus, using the examined weak-binding kinetic intermediates of the myosin ATPase cycle, the Triton X-100-induced delayed acto-HMM dissociation was not observed.

3.3. Effect of Triton X-100 on the HMM ATPase

To test whether Triton X-100 inhibited the HMM ATPase activity, thereby inhibiting in vitro motility,

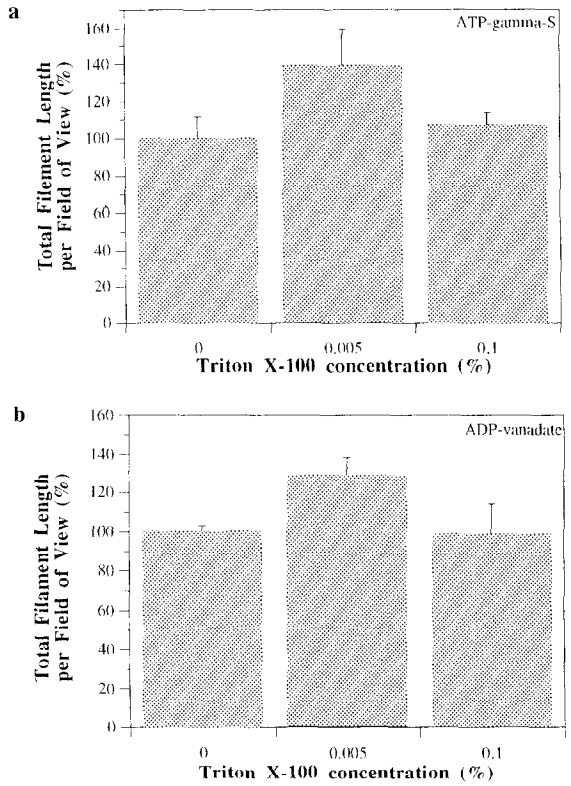

Fig. 7. Effect of kinetic intermediates on the Triton X-100-induced delayed acto-HMM dissociation. (a) Total length per field of view of actin filaments bound to HMM in the presence of 1 mM ATP–γ-S and increasing Triton X-100 concentration. (b) Total actin filament length per field of view of actin filaments bound to vanadate-trapped HMM, as a function of Triton X-100 concentration. Data are shown as mean ± SEM.

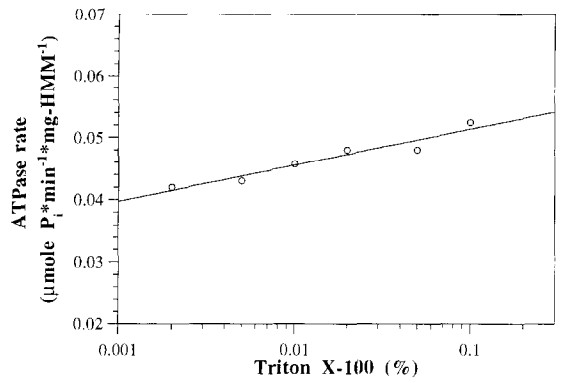

Fig. 8. Effect of increasing concentrations (in the range of 0.001–0.1%) of Triton X-100 on the actin-activated HMM ATPase activity.

the actin-activated HMM ATPase activity was measured at increasing concentrations of the detergent (Fig. 8). Triton X-100 was found not to decrease the ATPase activity of HMM; on the contrary, a slight increase in the ATPase activity was observed.

3.4. Factors affecting the Triton X-100-induced acto-HMM dissociation

The above observations indicate that the presence of actin filament sliding is important in evoking the Triton X-100-induced delayed acto-HMM dissociation. To probe the mechanisms of the phenomenon further, the acto-HMM dissociation delay time was measured under conditions where the actin filament sliding velocity was modified. ATP concentration and temperature have been previously described to affect sliding velocity [20,28]. By varying these parameters, the effect of V_{max} on the dissociation delay could be examined. Increasing the ATP concentration (at a Triton X-100 concentration of 0.005%) shortened the acto-HMM dissociation delay (Fig. 9a). At 30°C, the delay time decreased from 133.4 s at 0.05 mM ATP to 11.6 s at 4 mM ATP. Further increase in ATP concentration resulted in no resolvable delay in acto-HMM dissociation. Below 0.05 mM ATP, actin filaments continued to slide over the HMM-coated surface with no dissociation within the six minutes of observation. Similar results were observed at 20°C; the delay time decreased with increasing ATP concentration. The relationship between dissociation delay time and ATP concentration, however, was much steeper than at 30°C (Fig. 9a), possibly due to the high Q_{10} of both the sliding

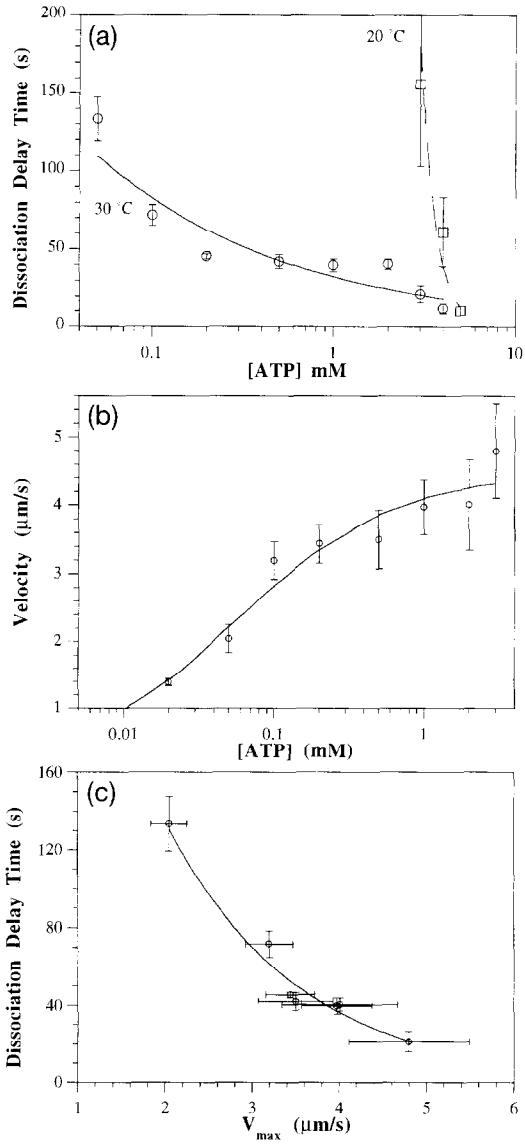

Fig. 9. In vitro actin filament motility over HMM in the presence of 0.005% Triton X-100 and varying concentrations of ATP. (a) Acto-HMM dissociation delay time as a function of ATP concentration. Data are shown as mean ± standard deviation for 20°C and 30°C. (b) Actin filament velocity as a function of ATP concentration. Data are shown as mean ± SEM. Temperature is 30°C. (c) Acto-HMM dissociation delay time as a function of maximal filament velocity prior to dissociation (V_{max}). Temperature is 30°C.

movement and the HMM ATPase. To examine the dissociation delay time as a function of V_{max}, the delay times measured at the different ATP concentrations were plotted against V_{max} measured at these ATP concentrations. Fig. 9b shows V_{max} as a function of ATP concentration. The dissociation delay time was plotted against the measured V_{max} on Fig. 9c. Dissociation delay time varied as an inverse function of V_{max}.

The acto-HMM delayed dissociation was also examined as a function of actin filament length. The dissociation delay time for individual actin filaments was plotted against their contour length (Fig. 10). Results are shown for acto-HMM delayed dissociation at three different ATP concentrations: 0.5, 3, and 4 mM. The dissociation delay time did not correlate with actin filament length.

3.5. Possible artifacts

The addition of the nonionic detergent Triton X-100 to the in vitro motility assay could in theory remove heavy meromyosin attached to the nitrocellulose surface. This could artificially lead to what was seen as actin filament dissociation from HMM. To test for this possibility, following the Triton-treatment, actin filaments were completely washed out of the sample chamber. Subsequently, a fresh sample of fluorescent actin filaments was added in the chamber, and allowed to bind to the surface. Upon the addition of ATP, actin filament motility began, indicating that the filaments were attached to the HMM on the surface, and not to the nitrocellulose. Furthermore, upon the addition of 0.005% Triton X-100 along with ATP, the delayed actin filament dissociation from HMM could be repeated.

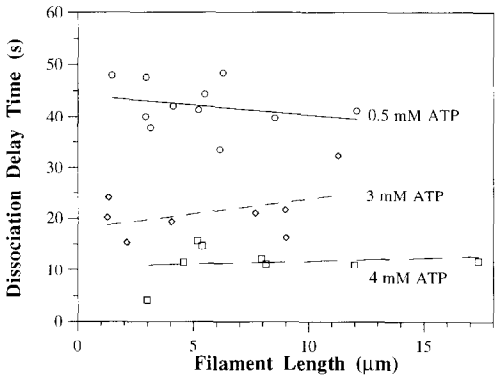

Fig. 10. Acto-HMM dissociation delay time as a function of actin filament length. Data are shown as mean ± SEM for in vitro motility observed at ATP concentrations of 0.5, 3 and 4 mM.

4. Discussion

Actin filament in vitro motility over HMM was studied in the presence of the nonionic detergent Triton X-100. Below 0.004% concentration, Triton X-100 did not affect the in vitro motility. Above 0.007%, actin filaments were dissociated from HMM, and motility could not be observed. In the concentration range of 0.004–0.007%, Triton X-100 induced the dissociation of the already sliding actin filaments from HMM. The dissociation occurred with a time delay, after which essentially all the actin filaments rapidly departed from the HMM-coated surface. The dissociation delay time decreased with increasing Triton X-100 concentration and increasing sliding velocity (due to increasing ATP concentration or increasing temperature), and was independent of actin filament length. In vitro acto-HMM binding assays showed that in the absence of ATP, Triton X-100 did not cause significant dissociation of the actin filaments from HMM. Further, the Triton X-100-induced delayed acto-HMM dissociation could not be seen in the case of weak-binding kinetic intermediates of the myosin ATPase, either. Thus, the nonionic detergent Triton X-100, in the concentration range of 0.004–0.007% causes a delayed, rapid, and complete dissociation of actin filaments from HMM, in the evoking of which the ATP-induced filament sliding is important.

The concentration range of Triton X-100 where the delayed acto-HMM dissociation occurred is below its critical micelle concentration (CMC, concentration at which detergent micelles begin to form). The CMC value of Triton X-100 is 0.29 mM (0.018%) [29]. In previous studies, Triton X-100 has been shown to inhibit the myosin K—ATPase [10]. The concentration at which Triton X-100 begins to have an effect is around its CMC value [10]. Since the delayed acto-HMM dissociation occurred at Triton X-100 concentrations a factor of 2–5 below

Triton's CMC value, it was reasonable to assume that the ATPase activity of myosin was not inhibited. In fact, the S-1 Mg—ATPase activity has been shown to be increased 1.3-fold at a Triton X-100 concentration of 0.1 mM [1]. Furthermore, in the present work it is shown that in the Triton X-100 concentration range of 0.001–0.1% the actin-activated HMM ATPase activity is indeed not reduced but slightly enhanced (Fig. 8). Thus, in the concentration range of Triton X-100 where the delayed acto-HMM dissociation occurs the ATPase activity is not reduced.

Triton X-100 caused not only a delayed acto-HMM dissociation, but also a reduction of the maximal sliding velocity prior to the dissociation (V_{max}). V_{max} decreased with increasing Triton X-100 concentration (see Fig. 4). The apparent reduction of V_{max} might partly be due to the partial dissociation of the sliding actin filaments of the HMM-coated surface. Such a partial dissociation was particularly evident in the case of long actin filaments which were sliding on the HMM-coated surface with segments wiggling above the surface. This might have contributed to the apparent reduction of sliding velocity. In support of this, when methylcellulose was added to the in vitro motility assay to reduce actin filament wiggling, high sliding velocities were sustained at Triton X-100 concentrations up to 0.01%. Thus, the reduced maximal actin filament sliding velocities were in part due to the partial filament dissociation present prior to the complete filament dissociation. While in the presence of methylcellulose motility was restored at Triton X-100 concentrations up to 0.01%, above this concentration movement eventually ceased. Since the ATPase activity was not reduced, it is possible that the absence of motility was caused by eventual acto-HMM dissociation, but the departure of actin filaments from the surface was not resolvable by fluorescence microscopy. That is, in spite of an effective dissociation of the actin filaments from the HMM surface filament diffusion did not occur due to the presence of methylcellulose.

The Triton X-100-induced delayed acto-HMM dissociation could be caused by a progressively reduced affinity of actin filaments to the HMM-coated surface. Once the affinity of actin filaments to HMM reaches a minimum value, the filament dissociates from the HMM-coated surface. Filaments are held to the surface by binding to the myosin heads. If the density of the myosin heads falls below a minimum, the sliding actin filaments dissociate from the surface [17]. Although in the experiments presented here the myosin head density was not changing (as evidenced by the reversibility experiments), it is conceivable that the number of myosin heads actually participating in holding and propagating the actin filament decreased upon the addition of Triton X-100. The methylcellulose experiments seem to support this idea: just as in the case of a sparsely myosin-coated surface [17], the addition of methylcellulose to the Triton-affected in vitro motility assay restored normal motility by reducing actin filament wiggling. The absence of correlation between actin filament length and dissociation delay time (Fig. 10), on the other hand, indicates that the delayed dissociation cannot be simply explained by the gradual decrease in the number of myosin heads holding the filament. In the case of such a gradual decrease the short actin filaments would dissociate from HMM sooner than the long ones. The concerted nature of the delayed dissociation, being independent from actin filament length, thus points to an underlying cooperative process. This hypothesis is supported by the steepness of the drop in dissociation delay time in response to a slight increase in Triton X-100 concentration.

Triton X-100 has previously been shown to reduce actomyosin affinity [1], possibly by binding to the hydrophobic residues located at the actomyosin interface [30]. This idea is supported by the reduced binding of F-actin to HMM pretreated with Triton X-100 (see Fig. 7). However, the mere binding of detergent molecules at the actomyosin interface does not explain the time-dependent (kinetic) nature of acto-HMM dissociation in the presence of ATP and 0.004–0.007% Triton X-100. The effect of Triton X-100 (delayed dissociation) seems to be associated with the time-dependent molecular processes undergoing in the in vitro motility assay. Such time-dependent processes include the ATPase activity of myosin (ATP molecules hydrolyzed as a function of time) and the sliding movement of the actin filament (spatial displacement as a function of time). The observation that the delayed acto-HMM dissociation was absent when the weak-binding M.ATP and M.ADP–P_i kinetic intermediates of the myosin ATPase cycle were used indicate that the time-dependent process

of myosin ATPase activity is not likely to be involved in the Triton-induced acto-HMM dissociation. Rather, the presence of actin filament sliding is important in evoking the delayed acto-HMM dissociation.

The acto-HMM delayed dissociation depends on the velocity of actin filament sliding. For a given Triton X-100 concentration, dissociation delay time was inversely proportional to sliding velocity (Fig. 10c). Thus, the rapid dissociation occurs after a certain distance has been travelled by the actin filaments. This distance, obtained as the multiple of the velocity and the delay time, is approximately 50–300 μm. The distance is independent of actin filament length, because the dissociation delay does not correlate with the length of the filaments. Thus, actin filaments of any size need to travel a certain distance over the HMM-coated surface prior to complete dissociation. Possibly, the sliding movement of the actin filament is associated with a conformational change in acto-myosin that increases the chance for Triton X-100 binding and hence leads to the progressive decrease of the myosin heads actually holding the filament. The eventual concerted actin filament dissociation may occur as a result of a cooperative process. In summary, the Triton X-100-induced delayed acto-HMM dissociation is likely to be associated with molecular events underlying the sliding/motility process.

There is a discrepancy between the dramatic effects of low concentrations of Triton X-100 in the in vitro motility assay and the lack of such in muscle fiber experiments. Possibly, the semi-crystalline lattice structure of the sarcomere is essential to maintain the contractile response of the muscle fiber in the presence of the detergent. Further work is needed to explore the molecular mechanisms underlying the differences in the response of the two systems to Triton X-100.

In the present work the nonionic detergent Triton X-100 was used to study the process of chemomechanical transduction in the experimental environment of the in vitro motility assay. The experimental approach revealed that a time-dependent actomyosin dissociation takes place upon the action of the detergent. Although nonionic detergents have previously been used in biochemical assays to study the myosin ATPase, their use in the in vitro motility assay may offer new insights into how chemical energy is converted to mechanical.

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