Effect of Mts1 on the Structure and Activity of Nonmuscle Myosin II†

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ABSTRACT: The *mts1* gene codes for a 9 kDa protein belonging to the S100 subfamily of Ca^{2+}-binding proteins and is known to play a role in metastasis. Its role in metastasis may be through cellular locomotion, as transfection of *mts1* into mouse mammary adenocarcinoma cells increases cellular motility in modified Boyden chemotaxis chambers. The Mts1 protein interacts with nonmuscle myosin II in the presence of Ca^{2+} with an affinity of approximately 7.9×10^4 M^{-1} and an approximate stoichiometry of 3 mol of Mts1/mol of myosin heavy chain. No interaction was found with myosin I or myosin V. The binding site of Mts1 on myosin is in the rod region, particularly to the light meromyosin portion of the rod. To understand the mechanism by which Mts1 alters cellular motility, we examined its effect on myosin structure and activity. Cosedimentation analysis and electron microscopy suggest that Mts1 destabilizes myosin filaments. In the presence of Ca^{2+}, Mts1 inhibits the actin-activated MgATPase activity of myosin *in vitro*. The data demonstrate an effect of Mts1 on both myosin structure and function, and suggest a route through which Mts1 affects motility as well as metastasis.

One of the leading causes of cancer related deaths stems from the ability of cancerous cells to leave the primary tumor, travel to distant sites, and form a secondary colony. Although the clinical significance of metastasis has been appreciated for years, very little is known about the molecular mechanisms which allow cells to become metastatic. A three-step theory of invasion proposed by Liotta *(1)* suggests that metastatic cells must be able to attach to components of the matrix via cell surface receptors, secrete hydrolytic enzymes to locally degrade the matrix, and then move into regions which were modified by proteolysis. To trigger such a multistep process, numerous gene products would have to be involved. Recently, a number of genes have been identified whose expression levels correlate with the metastatic phenotype *(2*−*8)*, including the *mts1* gene *(9)*.

The *mts1* gene encodes a 9 kDa protein that belongs to the highly conserved S100 subfamily (10) of Ca^{2+}-binding proteins. To date, there are 18 known S100 proteins *(11*−*13)*. Although the exact physiological roles of many of these are unknown, they have been implicated in such processes as microtubule dynamics *(14)*, cytoskeletal−membrane interactions *(15*−*17)*, cellular growth and differentiation *(17*−*21)*, calcium signal transduction pathways *(22)*, and chemotaxis *(23)*. Induction of the metastatic phenotype has been observed upon introduction of Mts1 into several different systems *(24, 25, 26, 27)*. The findings that nonmetastatic mouse mammary adenocarcinoma cells display increased motility upon expression of transfected *mts1 (28)* and that Mts1 binds nonmuscle myosin II *(29, 30)* suggest a mechanism through which Mts1 may play a role in metastasis.

It is conceivable that dysregulation of a gene product involved in the process of motility could enhance the metastatic phenotype of cancerous cells. Indeed, the expression of numerous proteins involved in motility is altered in tumorigenic and/or metastatic cells *(31*−*34)*. To understand the mechanism by which Mts1 influences cellular motility, we examined the effect of Mts1 on myosin structure and activity.

EXPERIMENTAL PROCEDURES

Purification of Proteins. Platelet myosin was purified as previously described *(35)*. Subfragment 1 (S-1)[1] and rod were prepared by papain digestion as described *(35)*. The rod portion of myosin was purified by ethanol precipitation. Platelet heavy meromyosin (HMM) and light meromyosin (LMM) were prepared by chymotrypsin digestion according to Sellers *et al. (35)*. A recombinant fragment of human nonmuscle myosin IIA, corresponding to an HMM-like fragment, was expressed in baculovirus *(36, 37)*. Rabbit skeletal muscle actin *(38)*, turkey gizzard myosin light chain kinase *(39)*, and bovine testes calmodulin *(40)* were prepared as described. Expression and purification of the glutathione *S*-transferase (GST)−human Mts1 fusion protein (GST−hMts1) were performed as described *(29)*. This fusion protein contains both a GST portion as well as a short amino acid sequence that can be recognized by the monoclonal FLAG antibody *(41)*.

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[1] Abbreviations: GST, glutathione *S*-transferase; GST−hMts1, GST−human Mts1; S-1, subfragment 1 of myosin; HMM, heavy meromyosin; LMM, light meromyosin.

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Gel Overlay Experiments. Gel overlay experiments were performed as described previously in the presence of 0.1 mM $CaCl_2$ *(29)* except that the myosins were electrophoresed on 8−10% SDS−polyacrylamide gels *(42)* and the filters were probed with ^{32}P-GST−human Mts1 (GST−hMts1) which was purified and labeled as described *(29)*. All samples were run in duplicate, and the duplicate filter was probed with ^{32}P-labeled GST or ^{32}P-GST−hMts1 in the absence of Ca^{2+} as a control. Samples for overlay were obtained through several different methods. HL-60 cells were lysed in RIPA buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1 mM EDTA, 0.5% sodium deoxycholate, 0.1% SDS, 25 μg/mL leupeptin, 40 μg/mL PMSF) to obtain the whole cell lysate, and platelet myosin was purified as described above. Purified myosin IIB, myosin I extract and recombinant HMM, and myosin V extract were gifts from Kazu Itoh, Fei Wang, and Nathalie Bonafe (NHLBI, NIH), respectively. Samples were electrophoresed on a 12.5% SDS−polyacrylamide gel and Coomassie stained prior to gel overlay experiments to determine approximate concentrations and integrities of the proteins.

Myosin Phosphorylation. Myosin in 25 mM NaCl, 10 mM MOPS, 10 mM $MgCl_2$, 0.1 mM EGTA, 3 mM NaN_3, and 1 mM DTT, pH 7.0, was phosphorylated by the addition of 0.2 mM $CaCl_2$, 1×10^{-7} M calmodulin, 1×10^{-8} M MLCK, and 1 mM ATP. The reaction was incubated at 25 °C for 15 min, and then stopped by the addition of 0.2 M NaCl and 0.5 mM EGTA.

Cosedimentation Analysis. Cosedimentation of Mts1 with myosin filaments was utilized to determine the Mts1-binding region of myosin as well as to determine the affinity of the Mts1−myosin interaction. Varying concentrations of purified proteins were added to a 200 μL ultracentrifuge tube in a 50 μL volume under low ionic strength conditions (50 mM NaCl, 1 mM $MgCl_2$, 1 mM MOPS, 0.1 mM EGTA, 3 mM NaN_3, pH 7.0) for 15 min at room temperature in the presence of 0.1 mg/mL bovine serum albumin (BSA), which was used as a volume marker for the pellets, and in the presence or absence of 0.2 mM $CaCl_2$. Samples were ultracentrifuged at 80 000 rpm, 25 °C for 10 min in a TLA100 rotor in the Optimal TLX ultracentrifuge (Beckman, Palo Alto, CA). The supernatants (40 μL) were then removed and added to 10 μL of 5× sample buffer (310 mM Tris, pH 6.8, 50% glycerol, 10% SDS) containing DTT and boiled. The pellets were resuspended in 25 μL of 1× sample buffer and boiled. The pellets and supernatants were electrophoresed on 12.5% SDS−polyacrylamide gels, stained with Coomassie blue, and quantitated using a Molecular Dynamics densitometer (Molecular Dynamics, Sunnyvale, CA). After correcting for the H_2O volume of the pellet by the amount of sedimented BSA, the amount of Mts1 bound to myosin was determined. The binding constant and stoichiometry of binding were calculated by fitting the data to the following equation: $b = B_{max}[S]/(K_D + [S])$.

Immunoprecipitation of Mts1 and Myosin, Phosphorylated Myosin, and HMM. Either platelet myosin, phosphorylated platelet myosin, or platelet HMM at a concentration of approximately 1.5 μM was mixed with 4.5 μM GST−hMts1 (containing a FLAG antibody-binding region) or with no Mts1 (negative controls) under low ionic strength conditions (20 mM NaCl and 75 mM KCl) in the presence of 0.4 mM $CaCl_2$. The samples were incubated on ice for 30 min, after which time they were mixed with the FLAG antibody and incubated overnight at 4 °C. A 1:1 slurry (20 μL) of protein A−Sepharose was then mixed with each sample and incubated 1 h at 4 °C. The beads were washed 3 times in wash buffer (50 mM NaCl, 10 mM MOPS, 10 mM $MgCl_2$, 0.1 mM EGTA, 1 mM DTT, 15 mM NaN_3, pH 7.0) plus 0.4 mM $CaCl_2$ and 0.2% Triton X-100. Samples were boiled in 2× Laemmli buffer and electrophoresed on 10% SDS−polyacrylamide gels.

Negative Staining Electron Microscopy. Formvar-coated electron microscope grids were carbon-coated and rendered hydrophilic by glow discharge in a Balzers carbon evaporation apparatus. Myosin filaments were prepared by dialysis against a buffer brought to 150 mM NaCl and preincubated with either GST−hMts1 or GST ± Ca^{2+}. Protein samples were applied to the glow-discharged grids for 30 s, and excess solution was blotted with filter paper and stained with 1% uranyl acetate solution for 15 s as described by Niederman and Pollard *(43)*. Electron micrographs were taken on a JEOL 100CX electron microscope.

ATPase Assays. Platelet myosin filaments were prepared by dialysis against a buffer of 150 mM NaCl, 10 mM MOPS, 10 mM $MgCl_2$, 0.1 mM EGTA, 3 mM NaN_3, and 1 mM DTT, pH 7.0. Myosin and HMM were phosphorylated as described above, but with the addition of 0.5 mM [^{32}P]ATP instead of 1 mM cold ATP. For myosin, the MgATPase assay was carried out at 37 °C in 50 mM KCl, 10 mM MOPS, 0.1 mM EGTA, 5 mM $MgCl_2$, and 1 mM [^{32}P]ATP. For HMM, the MgATPase was performed in 13 mM MOPS, 0.11 mM EGTA, 2 mM $MgCl_2$, and 1 mM [^{32}P]ATP at 37 °C. Protein concentrations in respective assays were as follows: 0.13 μM myosin, 0.09 μM HMM, 10 μM F-actin, 2 μM GST, 2 μM GST−hMts1. Depending on different reaction conditions, 0.2 mM $CaCl_2$ or 0.5 mM EGTA were included. Aliquots were removed from the reaction at time intervals of 20, 40, 60, and 80 min, and ATPase activity was measured by the release of P_i *(44)*.

RESULTS

Mts1 Interacts with Nonmuscle Myosin II Isoforms A and B, but Not with Myosin I or Myosin V. Previous studies have demonstrated an interaction of Mts1 with nonmuscle myosin II, but not with smooth muscle myosin *(29, 30)* or skeletal muscle myosin *(29)*. A large multigene family of myosin proteins exists, which includes 2 isoforms of the conventional nonmuscle myosin II (termed A and B) as well as various isoforms from at least 11 other classes *(45)*. We now show that Mts1 interacts with both nonmuscle myosin II isoforms, but not with proteins from two other classes of myosin, myosins I and V. To demonstrate which isoforms Mts1 interacts with, a nylon filter containing various isoforms and classes of myosin was probed with ^{32}P-labeled GST-human Mts1 (GST−hMts1)[1] in the presence of 0.1 mM $CaCl_2$. Figure 1, lane 1, serves as a positive control, as it had previously been shown that Mts1 binds nonmuscle myosin II from HL-60 cells *(29)*. Figure 1, lanes 2 and 3, demonstrates that GST−hMts1 binds both A and B isoforms of nonmuscle myosin II, respectively. However, it does not bind myosin I or myosin V (lanes 4 and 5, respectively). In addition, a second filter containing the same concentrations of the different myosins was probed with ^{32}P-labeled GST as a control. The GST probe did not bind to any of the myosins on the filter (data not shown).

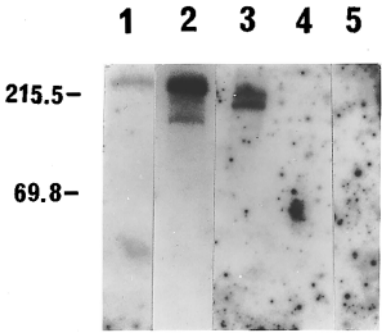

FIGURE 1: Mts1 binds nonmuscle myosin II isoforms A and B, but does not bind myosin I or myosin V. A nylon filter containing several different types of myosin was probed with GST−hMts1 as described under Experimental Procedures. (1) Whole cell lysate from HL-60 cells, (2) purified platelet myosin (nonmuscle myosin IIA), (3) myosin IIB immunoprecipitated from Y-79 retinoblastoma cells, (4) partially purified recombinant myosin I extract from baculovirus cells, (5) partially purified chicken brain myosin V extract.

Mts1 Binds the Rod of Nonmuscle Myosin II in the LMM Region. We next investigated where the Mts1 binding site on myosin II was located. Purified platelet myosin (myosin IIA) was digested with papain to prepare the subfragment 1 (S-1) and the rod fragments of myosin. S-1 is a single-headed molecule containing both light chains and binding sites for ATP and actin *(35)*, but is lacking the tail region. The rod is composed of the two heavy chains of the tail in a coiled-coil structure which retains the ability to self-associate into filaments. The two fragments of myosin were then purified, and gel overlay experiments were performed as described under Experimental Procedures. Gel overlay experiments in the presence of Ca^{2+} suggested that Mts1 binds the rod portion of myosin, but not the S-1 fragment (data not shown). To confirm an interaction of Mts1 with the rod of myosin, GST−hMts1 was cosedimented with the myosin rod in the presence of Ca^{2+}. Figure 2A and Figure 2B are control cosedimentation experiments. Figure 2A demonstrates that GST does not cosediment with platelet myosin in the presence or absence of Ca^{2+} (panel A), providing evidence that it is the Mts1 protein and not the GST portion of the fusion protein that binds myosin. In Figure 2, the Ca^{2+} dependence of the interaction is confirmed (panel B, lanes 1, 2, 4, and 5), and evidence is provided that GST−hMts1 does not sediment in the absence of platelet myosin (panel B, lanes 3 and 6). GST−hMts1 is observed to cosediment with the rod region of nonmuscle myosin II (Figure 2C, lane 1), whereas in the absence of rod (Figure 2C, lane 2) Mts1 does not sediment. Lanes 3 and 4 are the respective supernatants. The BSA present is added as an internal control to measure the water volume of the pellet. The cosedimentation analysis confirms that Mts1 interacts with the rod portion of nonmuscle myosin II.

To determine if Mts1 binds the heavy meromyosin (HMM) fragment or the light meromyosin fragment (LMM) of the myosin rod, fragments were prepared by chymotryptic digestion of platelet myosin, and coimmunoprecipitations were performed. The HMM fragment contains both myosin heads and the coiled-coil subfragment-2. It is soluble at low ionic strength and binds actin, hydrolyzes MgATP, and translocates actin filaments at rates comparable to undigested myosin *in vitro*. In contrast, the LMM fragment contains the C-terminus of the rod, and is able to remain in the coiled-coil structure and to assemble into filament-like aggregates

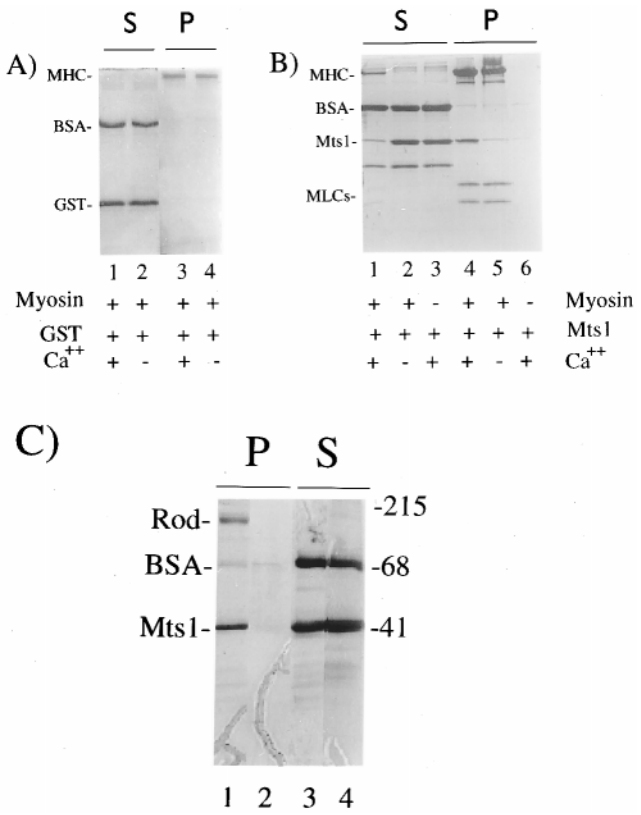

FIGURE 2: Mts1 binds the rod region of nonmuscle myosin II. (A) 12.5% SDS−polyacrylamide gel of a control cosedimentation experiment demonstrating that GST does not interact with myosin in the presence or absence of Ca^{2+}. Lanes 1 and 2 are the supernatants; lanes 3 and 4 are the pellets from samples containing 5 μM platelet myosin, 5 μM GST, and 0.2 mM Ca^{2+} (lanes 1 and 3), or the same concentrations of myosin and GST in the absence of Ca^{2+} (lanes 2 and 4). Note that in both cases the GST remains in the supernatant. (B) 12.5% SDS−polyacrylamide gel of a control experiment demonstrating that the interaction of GST−hMts1 and myosin is Ca^{2+}-dependent in cosedimentation analyses, and that GST−hMts1 does not sediment in the absence of myosin. Lanes 1−3 are the supernatants; lanes 4−6 are the pellets from reactions containing 1.3 μM platelet myosin, 4.2 μM GST−hMts1, and 0.2 mM Ca^{2+} (lanes 1 and 4), the same concentrations of platelet myosin and GST−hMts1 in the absence of Ca^{2+} (lanes 2 and 5), and GST−hMts1 alone (lanes 3 and 6). The band just beneath GST−hMts1 is believed to be a breakdown product of Mts1. (C) Cosedimentation demonstrates an interaction of the myosin rod with GST−hMts1. Lanes 1 and 2 are the pellets from each reaction, and lanes 3 and 4 are the supernatants. Lanes 1 and 3 are rod, excess GST−hMts1, and 0.2 mM Ca^{2+}, and lanes 2 and 4 are the same concentration of GST−hMts1 as in lanes 1 and 3 and 0.2 mM Ca^{2+}. All reactions contain BSA as an internal control. (MHC) myosin heavy chain, (Mts1) GST−hMts1, (MLCs) myosin light chains.

(45). Coimmunoprecipitations were performed in the presence of Ca^{2+} and GST−hMts1 and myosin, HMM, or LMM with an antibody against the FLAG peptide found in the GST−hMts1 fusion protein. In addition, immunoprecipitations were performed with each protein alone to moniter background levels of precipitation in the absence of GST−hMts1 (data not shown). Because myosin must be phosphorylated to have MgATPase activity, we investigated the binding of GST−hMts1 to phosphorylated myosin by coimmunoprecipitation. GST−hMts1 bound platelet myosin and phosphorylated platelet myosin, but not HMM (Figure 3A). Under these ionic conditions, LMM forms sedimentable aggregates, and we were therefore unable to determine whether Mts1 bound LMM. To circumvent this problem,

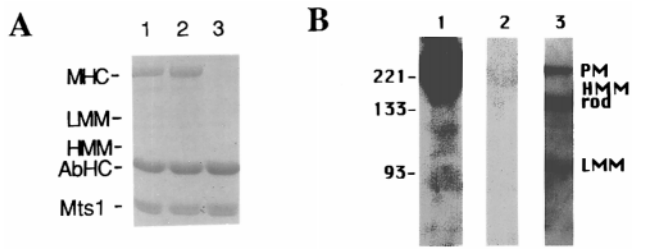

FIGURE 3: Mts1 binds both phosphorylated and nonphoshorylated myosin, and specifically binds the LMM region of the myosin rod. (A) Mts1 was coimmunoprecipitated with myosin, phosphorylated myosin, or HMM as described under Experimental Procedures. Coimmunoprecipitates of Mts1 and (1) platelet myosin, (2) phosphorylated platelet myosin, and (3) HMM. The proteolytically prepared HMM for this experiment is cleaved 65 kDa from the amino terminus and migrates as a closely spaced doublet at approximately 65 kDa (marked in figure). (B) A nylon filter containing (1) whole platelet myosin, (2) recombinant HMM, and (3) a chymotryptic digest of myosin containing an LMM fragment was probed with GST−hMts1 as described under Experimental Procedures. The recombinant HMM used in this experiment migrates at approximately 150 kDa (marked in figure).

we performed gel overlay experiments on a chymotryptic digestion of platelet myosin containing LMM as well as on a sample of recombinant HMM. The gel overlay in Figure 3B demonstrates the binding of Mts1 to whole platelet myosin and LMM, but not to HMM.

Binding of Mts1 to Myosin. To determine an approximate affinity of the Mts1−myosin interaction, 3 μM platelet myosin was cosedimented with GST−hMts1 ranging from 1 to 9 μM in the presence of Ca^{2+}. After centrifugation, pellets and supernatants were electrophoresed on 12.5% SDS−polyacrylamide gels, and the amount of each protein pelleted vs that remaining in the supernatant was determined by densitometric scanning. Binding curves constructed from the data of several experiments revealed an approximate K_d of 12.6 μM and an approximate stoichiometry of 3 mol of Mts1 bound/1 mol of myosin heavy chain (data not shown).

Mts1 Appears To Destabilize Myosin Filaments. It was observed that as increasing concentrations of GST−hMts1 were added to the cosedimentation reaction, myosin no longer completely sedimented. Figure 4 demonstrates the appearance of the myosin heavy chain in the supernatant upon addition of GST−hMts1. Note that with increasing concentrations of GST−hMts1, more cosedimentation of GST−hMts1 with myosin is observed, but additionally, more myosin is found in the supernatant. This result was unexpected since under these salt and sedimentation conditions, myosin is known to be filamentous and to pellet *(46)*. The fact that the myosin heavy chain appears in the supernatant in the presence of GST−hMts1 suggests that GST−hMts1 may destabilize myosin filaments. Additionally, this effect is enhanced in the presence of ATP (data not shown), which favors depolymerization of dephosphorylated myosin filaments *(46)*.

Negative Staining Electron Microscopy Demonstrates Altered Myosin Filament Structure in the Presence of Mts1. To determine the effect of Mts1 on myosin filaments, myosin structure was examined by negative staining electron microscopy. Myosin filaments in samples with Ca^{2+} Figure 5, panel A), with GST and Ca^{2+} (data not shown), and with GST−hMts1 in the absence of Ca^{2+} (Figure 5, panel D) had a similar appearance as myosin alone (data not shown). These filaments appeared as a homogeneous population of short filaments with tapering ends. In contrast, in the samples with GST−hMts1 and Ca^{2+} (Figure 5, panels B and C, which represent 5 min and 45 min preincubations of myosin in the presence of GST−hMts1 and Ca^{2+}, respectively), considerable background material which may correspond to monomeric myosin was observed. The length of incubation did not drastically alter the results, suggesting that Mts1 and myosin associate fairly rapidly.

MgATPase Activity of Platelet Myosin Is Inhibited in the Presence of Mts1. Data demonstrating the effect of Mts1 on the actin-activated MgATPase activity of platelet myosin are shown in Figure 6A. Myosin's actin-activated MgATPase activity is only very slightly inhibited in the presence of GST or in the presence of GST−hMts1 without Ca^{2+} (conditions where Mts1 does not bind myosin). In contrast, when GST−hMts1 plus Ca^{2+} is added to the MgATPase assay, actin-activated myosin MgATPase is 88% inhibited.

Since evidence in the literature suggests that Mts1 may also bind actin in a Ca^{2+}-dependent manner *(47)*, we were interested in whether the inhibition of myosin's MgATPase activity was due specifically to Mts1 binding to myosin, or whether this inhibition could be conferred through binding to actin. Therefore, ATPase assays were performed with HMM, which retains actin-activated MgATPase activity but does not bind Mts1. GST−hMts1 plus Ca^{2+} results in only a very slight inhibition of HMM's actin-activated MgATPase activity (18%) (Figure 6B). This suggests that Mts1 acts by binding myosin to inhibit myosin ATPase activity, and

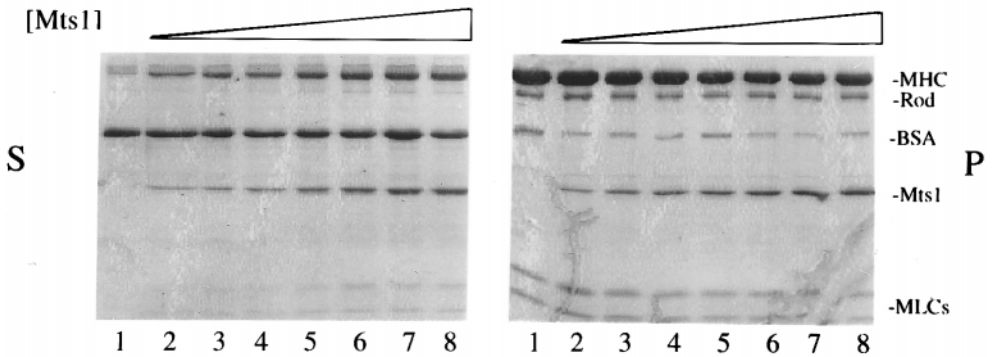

FIGURE 4: Cosedimentation analysis suggests that destabilization of platelet myosin filaments occurs in the presence of Mts1 and Ca^{2+}. Cosedimentation analyses were performed as described under Experimental Procedures. 12.5% SDS−PAGE of supernatants (S, left panel) and pellets (P, right panel) from samples containing 3 μM myosin only (lane 1) or 3 μM myosin plus increasing concentrations of GST−hMts1 ranging from 1 to 7 μM (lanes 2−8). All reactions contain BSA as an internal volume marker for the pellet.

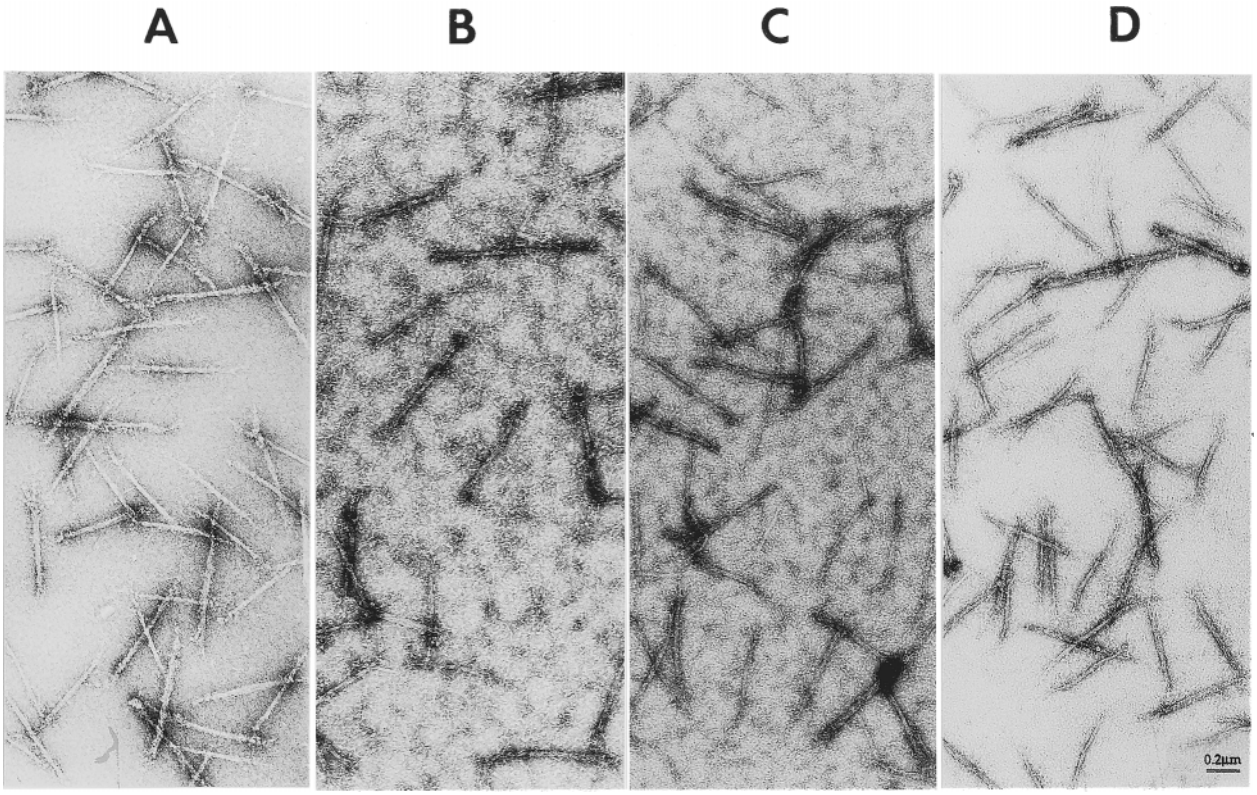

FIGURE 5: Mts1 in the presence of Ca^{2+} alters myosin filament structure. Negatively stained electron micrographs were prepared as described under Experimental Procedures. (A) Myosin in the presence of Ca^{2+}. (B) Myosin in the presence of GST−hMts1 and Ca^{2+}, 5 min preincubation. (C) Myosin in the presence of GST−hMts1 and Ca^{2+}, 45 min preincubation. (D) Myosin in the presence of GST−hMts1, no Ca^{2+}. The concentration in all samples was 1.4 μM myosin and 4.4 μM GST−hMts1. The $CaCl_2$ concentration was 0.2 mM when present.

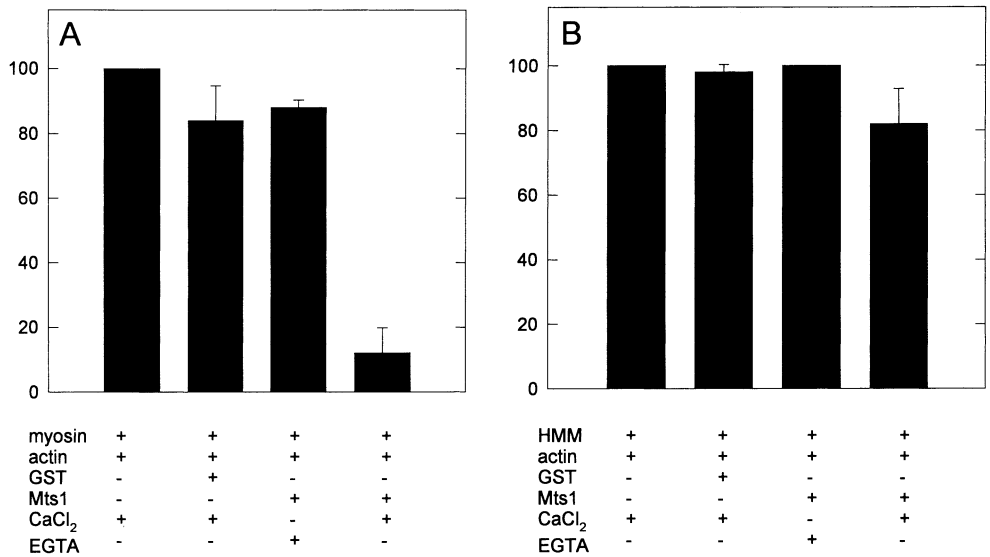

FIGURE 6: Mts1 in the presence of Ca^{2+} inhibits actin-activated myosin MgATPase activity by 88%, but only slightly inhibits actin-activated HMM MgATPase activity. (A) MgATPase of myosin. (B) MgATPase of HMM. ATPase assays were preformed as described under Experimental Procedures. Panel A is the average of 5 myosin MgATPase assays; panel B is the average of 4 HMM MgATPase assays. Wild-type myosin MgATPase rate: 0.19 ± 0.03 mol of ATP (mol of myosin)$^{-1}$ s^{-1}. Wild-type HMM MgATPase rate: 0.13 ± 0.04 mol of ATP (mol of myosin)$^{-1}$ s^{-1}.

that this inhibition is not solely through its interaction with actin.

DISCUSSION

Mts1 Binds the Rod of Myosin's IIA and B, but Not Myosin I or V. Gel overlay experiments demonstrate that Mts1 binds nonmuscle myosin II isoforms A and B, but does not bind myosin I or myosin V. The A and B isoforms of nonmuscle myosin are highly conserved. Chicken nonmuscle myosin II isoform A is 87% and 72% conserved to the head and tail portion, respectively, of chicken nonmuscle myosin II isoform B *(48)*. In contrast, nonmuscle myosin II differs substantially from myosin I and myosin V. Although the head regions of the various myosins are fairly conserved, the tail regions are strikingly different. The myosin II tail consists of dimerized heavy chains that form a parallel

α-helical coiled-coil through which the myosin self-associates to form filaments. Myosin I lacks the heptad repeats characteristic of coiled-coils, and is therefore incapable of dimerization *(45, 49)*. Myosin V has a tail which is a mixture of coiled-coil and globular domains *(50)*, but there is no evidence that the coiled-coil region self-associates into filaments *(51)*. Because the tails of the various myosins are distinct, and because Mts1 binds both myosin II isoforms but not myosin I or myosin V, we reasoned that Mts1 may bind the tail (rod) region of myosin II. Indeed, Mts1 was found to bind the rod portion of myosin II. Further analysis demonstrates that Mts1 binds the latter portion of the myosin rod (LMM), rather than HMM, which carries the proximal portion of the coiled-coil region.

Mts1 Binds Myosin II with an Approximate K_d of 12.6 μM and May Destabilize Myosin Filaments. Cosedimentation analysis at 50 mM NaCl revealed an approximate K_d for GST−hMts1 and myosin heavy chain of 12.6 μM. This affinity is comparable to that of other myosin binding proteins such as kinase-related protein (KRP or telokin), caldesmon, and myosin light chain kinase (MLCK) *(52−54)*. The affinity for GST−hMts1 and myosin utilizing cosedimentation analysis may be higher than calculated, since only the portion of GST−hMts1 which cosedimented with myosin was considered bound, whereas all GST−hMts1 in the supernatant was considered free. However, since it appears that GST−hMts1 destabilizes myosin filaments (as demonstrated by cosedimentation analysis as well as electron micrographs), causing some myosin to remain in the supernatant following sedimentation, we cannot be certain whether all the GST−hMts1 in the supernatant is free, or if a portion is bound to the soluble myosin.

To date, no proteins have been identified that destabilize myosin filaments. However, proteins that stabilize myosin filaments are known; i.e., telokin *(54)* and caldesmon *(55)*. In addition to Mts1, several other S100 proteins have been implicated in assembly/disassembly of proteins. In particular, $S100a_0$ stimulates the disassembly of desmin intermediate filaments in the presence of micromolar levels of Ca^{2+} *(56)*. Glial fibrillary acidic protein (GFAP) assembly/disassembly is also affected by S100 proteins in a Ca^{2+}-dependent manner, where calpactin I (which contains two copies of an S100-like protein, p11) stimulates assembly *(56−58)* and S100 protein stimulates disassembly *(59, 60)*. Taken together, this suggests that S100 proteins may be important in regulating cytoskeletal dynamics.

How Mts1 destabilizes myosin is not known. By comparing Figures 2 and 4, it appears that Mts1 destabilizes whole myosin filaments, but not rod filaments. There has always been the perplexing finding that myosin will fold into a 10S conformation but that rod will not and this is likely due to an ATP-dependent myosin rod binding site located near the regulatory light chain binding site (*61*) that is absent from rod preparations. It is also known that some monoclonal antibodies that bind near the C-terminus of myosin tend to disrupt myosin filaments. It is possible that Mts1 has a similar action on myosin (i.e., disrupts filaments) and that depolymerization is augmented by myosin's tendency to form 10S structures.

Mts1 Inhibits Myosin MgATPase Activity. It is known that Mts1 binds actin and tropomyosin in addition to myosin *(29, 30, 47, 62)*, and Mts1 has been colocalized with both myosin *(30)* and actin *(47)*. Our data demonstrate that GST−hMts1 inhibits the actin-activated MgATPase activity of myosin through its interaction with myosin. Two other cytoskeletal proteins known to inhibit actin-activated myosin MgATPase are caldesmon and calponin (see review *63*, *64*). Interestingly, both these proteins also bind myosin and actin, as well as tropomyosin. However, both calponin and caldesmon appear to inhibit myosin MgATPase activity via actin *(65, 66)*. Some models for caldesmon inhibition propose a tethering of myosin to actin, leading to a nonproductive interaction between the two proteins *(67)*. While our data suggest that the primary inhibition of the myosin MgATPase is via the interaction with myosin, we cannot rule out some actin involvement. However, we show that GST−hMts1 does not interact with HMM and does not significantly inhibit the actin-activated MgATPase of HMM, demonstrating that GST−hMts1 does not inhibit solely through actin. The mechanism by which GST−hMts1 inhibits myosin MgATPase activity is still unclear. It is possible that the destabilization of myosin filaments could lead to decreased MgATPase activity. Alternatively, Mts1 may be tethering myosin and actin in such a way that actin cannot properly activate myosin ATPase activity, as described above.

Overexpression of Mts1 in a mouse mammary adenocarcinoma cell line (CSML0) led to an increase in cell motility consistent with the phenotype of metastatic cells *(28)*. However, our results show that GST−hMts1 inhibits *in vitro* myosin MgATPase activity and appears to destabilize myosin filaments. These apparently contrary observations can possibly be reconciled by the known complexity of the mechanisms involved in cell locomotion, where it is necessary to rapidly assemble and disassemble myosin and actin filaments in different areas of the cell. Elucidating the role of Mts1 in cellular motility will help us not only to understand normal cellular motility, but also to gain greater insight into metastasis, a process with great clinical significance, about which relatively little is still known.

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