**Selective Removal of the Carboxyl-terminal Tail End of the Dictyostelium Myosin II Heavy Chain by Chymotrypsin**

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**Dictyostelium** myosin II is a conventional myosin consisting of two heavy chains of 243,000 Da and two pairs of light chains of 16,000 and 18,000 Da. In this paper, we show that the heavy chain of myosin II can be rapidly and selectively cleaved by chymotrypsin to yield two fragments with molecular weights of 195,000 and 38,000 Da as estimated from sodium dodecyl sulfate-polyacrylamide gels. Chymotryptic cleavage at this site occurs most readily in the absence of salt and is greatly inhibited as the salt concentration is increased from 0 to 60 mM. Amino acid sequence analysis of the small fragment demonstrates that its amino terminus corresponds to lysine 1826 of the myosin II heavy chain. If the fragment extends to the carboxy terminus of the myosin II heavy chain, it would have a molecular weight of 35,700. Upon digestion of myosin II which has been phosphorylated with a high molecular weight Dictyostelium myosin heavy chain kinase (Côté, G. P., and Bukiewicz, U. (1987) J. Biol. Chem. 262, 1065–1072), all of the phosphate is recovered on the 33,700-Da tail-end fragment. Chymotrypsin-cleaved myosin II is shown to be capable of forming filaments at salt concentrations between 20 and 100 mM as judged by its ability to be sedimented by centrifugation. Only the large fragment of myosin II is found in the pellet; the 33,700-Dalton fragment remains soluble. Chymotrypsin-cleaved myosin II can bind to actin and displays a high Ca²⁺-activated ATPase activity but has very low actin-activated ATPase activity.

The lower eukaryote *Dictyostelium discoideum* contains at least two distinct enzymes with myosin-like enzymatic and actin-binding properties. The smaller of these has a native molecular weight of about 150,000 (1) and seems to be similar to the single-headed myosin I enzymes first identified in *Acanthamoeba* (2, 3). The role played by this small myosin in cell motility is not yet elucidated. The larger enzyme (myosin II) is structurally similar to other muscle and nonmuscle myosins and consists of two heavy chains, each of 243 kDa (4), and two pairs of light chains, of 16 and 18 kDa, arranged in a highly asymmetric molecule possessing two globular heads and a 180-nm-long α-helical coiled-coil tail (5, 6). *In vivo*, Dictyostelium myosin II is phosphorylated both on the heavy chain and the 18-kDa light chain (7). The levels of phosphate on both the heavy and light chains transiently increase when *Dictyostelium* amoebae are stimulated by the chemoattractant cyclic AMP (8–10). The time course of the increase in myosin phosphorylation correlates with a shape change in the amoeba (9) and with a rapid, reversible reorganization of the myosin II filaments within the Dictyostelium (11). It is suggested then that chemoattractive stimulation of Dictyostelium alters the activity of specific myosin II kinases that in turn regulate the filament-forming properties and contractile activity of myosin II (reviewed in Ref. 12).

To date, most studies have concentrated on the role of heavy chain phosphorylation in regulating the properties of Dictyostelium myosin II. Peptide mapping seems to indicate that multiple sites on the Dictyostelium myosin II heavy chain are phosphorylated (19), and indeed several forms of Dictyostelium myosin II heavy kinases have been isolated. Kuczmaszki (13) has identified a myosin II heavy chain kinase from Dictyostelium which phosphorylates serine and threonine residues, while Maruta et al. (14) have partially purified two kinases, both with a molecular weight of 70,000 as estimated by gel filtration, which phosphorylate only threonine residues. We have purified to near homogeneity a Dictyostelium myosin heavy chain kinase with a molecular weight of 130,000 as estimated by SDS-polyacrylamide gel electrophoresis, but of greater than 700,000 as determined by gel filtration, which seems to phosphorylate a single threonine residue on each of the myosin II heavy chains (15). All of the kinases phosphorylate the tail region of myosin II and inhibit the actin-activated ATPase activity of the myosin (13–17).

To better understand the means by which heavy chain phosphorylation regulates the properties of Dictyostelium myosin II, it will be necessary to first define more exactly the number and location of the phosphorylation sites on the heavy chain and then to gain some information on conformational or structural changes brought about in the myosin heavy chain by phosphorylation. In this paper, we demonstrate that a specific site toward the carboxyl terminus of the Dictyostelium myosin II heavy chain is extremely susceptible to chymotryptic digestion at low salt concentrations. Selective removal of the end of the myosin II tail, which is shown to contain the threonine residue phosphorylated by the 700-kDa myosin II heavy chain kinase, does not alter the Ca²⁺-ATPase activity of myosin II but does inhibit actin-activated ATPase activity. The chymotrypsin-cleaved myosin II retains the ability to bind to actin and form filaments.

**Experimental Procedures**

[γ-32P]ATP was from ICN. ATP, imidazole (grade III), phenylmethylsulfonyl fluoride, Nα-p-tosyl-L-lysine chloromethyl ketone-treated chymotrypsin, and bovine serum albumin standard for the Bradford assay were from Sigma. Sepharose CL-4B was from Pharmacia Biotechnology, Inc. The reagent for the Bradford assay and chemicals for polyacrylamide gel electrophoresis were from Bio-Rad.

Preparation of Proteins—*D. discoideum* strain Ax-3 (American Institute for Biological Sciences, Urbana, Ill.) wild-type axenic amoebae were used. Axenic amoebae were grown in liquid medium supplemented with 50 μg/ml thiamine and 5% charcoal-stripped horse serum in the presence of 20 μM 6-amino purine (18). At 5 days postinoculation, the myoblasts were collected by centrifugation and resuspended in 10 mM Tris–HCl, pH 6.8, containing 0.1% NaN₃, 0.5 M proline, and 1 mM phenylmethylsulfonyl fluoride (PMSF, catat. 63606, Sigma Chemical Co., St. Louis, MO). The resulting cell suspension was homogenized using a Dounce homogenizer, and the homogenate was centrifuged at 300,000 × g for 2 h.

The abbreviations used are: SDS, sodium dodecyl sulfate.
Type Culture Collection) was grown and harvested as described previously (1). Dictyostelium myosin II and the myosin II heavy chain kinase were purified as described (15) with the following changes. The myosin II fractions recovered from the Sepharose CL-4B column were not passed over a Dowex 1-X2 column but were dialyzed immediately against 0.1 M KCl, 20% sucrose, 10 mM imidazole, 1 mM dithiothreitol, pH 7.0 buffer. Elimination of the Dowex column step had no detectable effect on the properties of the myosin. The purification of the myosin II heavy chain kinase involved the steps up to and including the hydroxylapatite column. The kinase was stored on ice in 0.1 M KCl, 50% sucrose, 10 mM imidazole, 2 mM dithiothreitol, pH 7.0, and used in this form.

Phosphorylation of Myosin II—Dictyostelium myosin II, dialyzed into 30 mM KCl, 10 mM imidazole, 1 mM MgCl₂, 1 mM dithiothreitol, pH 7.5, was pelleted by centrifugation in a Ti-50 rotor (40,000 rpm for 20 min) and resuspended in 10 mM imidazole, 2 mM MgCl₂, 1 mM dithiothreitol, and 0.5 mM [γ-32P]ATP (50 Ci/mmol). One ml of the myosin solution (1.3 mg/ml) was phosphorylated at 25 °C by the addition of 0.6 M KCl and 5 mM EDTA, and the myosin was then separated from the kinase and ATP by passage over a 1 x 30-cm Sepharose CL-4B column equilibrated in 0.6 M KCl, 10 mM imidazole, and 1 mM dithiothreitol. The myosin, containing 2 mol of phosphate/mol, was dialyzed overnight against 10 mM imidazole, 1 mM dithiothreitol, and used within 1 week.

Chymotryptic Digestion of Myosin II—Dictyostelium myosin II was dialyzed into 10 mM imidazole, 1 mM dithiothreitol, pH 7.5 buffer, and then diluted with this buffer to a protein concentration of 0.2-0.3 mg/ml. For experiments at higher ionic strengths, the diluting buffer contained the appropriate concentration of NaCl. Unless stated otherwise, a chymotrypsin to myosin ratio (w/w) of 1:100 was used for digestions at 25 °C, and a ratio of 1:40 was used at 0 °C. Digestions were performed with an Applied Biosystems gas phase sequencer and were not passed over a Dowex 1-X2 column but were dialyzed immediately against 0.1 M KCl, 20% sucrose, 10 mM imidazole, and 1 mM dithiothreitol. The kinase was stored on ice in 0.1 M KCl, 50% sucrose, 10 mM imidazole, 2 mM dithiothreitol, pH 7.0, and used in this form.

RESULTS

Chymotryptic Digestion of Dictyostelium Myosin II—The 245-kDa heavy chain of Dictyostelium myosin II can be rapidly and selectively cleaved by chymotrypsin to yield two fragments with molecular masses, as estimated from SDS-polyacrylamide gels, of 195 and 38 kDa (Fig. 1). When the digestion is carried out at 25 °C, using a chymotrypsin to myosin ratio (w/w) of 1:100, cleavage of the myosin heavy chain is complete by 10 min; however, loss of the 38-kDa fragment into lower molecular mass peptides is observed (Fig. 1, lanes A–D). The cleavage of the myosin heavy chain into the 195- and 38-kDa fragments occurs rapidly even when carried out on ice (Fig. 1, lanes A–D). At a chymotrypsin to myosin ratio (w/w) of 1:40, digestion of the myosin heavy chain is virtually complete by 0.5 min, and little further degradation of either of the cleavage products occurs for several minutes.

Dictyostelium myosin II isolated as described under "Experimental Procedures" has previously been shown to contain little covalently bound heavy chain phosphate, and we will refer to this myosin as "unphosphorylated myosin II" (15). A 700-kDa Dictyostelium kinase that phosphorylates only threonine residues (15) was used to incorporate 1 mol of phosphate into each of the Dictyostelium myosin II heavy chains. Phosphorylation of the myosin does not seem to significantly alter the rate at which chymotrypsin cleaves the myosin heavy chain, and the digestion products, once produced, remain stable for several minutes (Fig. 2). However, in addition to the 195- and 38-kDa fragments, a minor cleavage product of 40 kDa is also evident. The 40-kDa fragment is observed when unphosphorylated myosin II is digested at 25 °C but does not seem to be produced when the digest is performed at 0 °C (Fig. 1). An autoradiogram of the SDS-polyacrylamide gel demonstrates that all of the phosphate incorporated into the myosin heavy chain ends up in the 38- and 40-kDa fragments, and none of it remains associated with the large 195-kDa fragment (Fig. 2).

Optimal Conditions for Selective Cleavage of the Myosin II Heavy Chain—The specific hydrolysis of the Dictyostelium myosin II heavy chain into the 195- and 38-kDa fragments by chymotrypsin occurs only under certain conditions. The susceptibility of this site to chymotrypsin was found not to be dependent on the myosin concentration (0.15–1.5 mg/ml) or the sucrose concentration (0–20%) in the digestion mixture (data not shown), but was greatly reduced as the ionic strength increased (Fig. 3). Under conditions where almost complete cleavage of the myosin II heavy chain occurs in the absence of NaCl, virtually no cleavage is observed in 0.1 M NaCl (Fig. 3). Chymotryptic cleavage of the heavy chain remains inhibited when the NaCl concentration is increased to 0.2 M.

Quantitative densitometric scanning of SDS-polyacryl-
Fig. 2. Chymotryptic digestion of phosphorylated Dictyostelium myosin. Dictyostelium myosin II was phosphorylated as described under "Experimental Procedures" and digested at a protein concentration of 0.19 mg/ml with chymotrypsin at 0 °C. Lanes A–D represent a Coomassie Blue-stained, 8% SDS-polyacrylamide gel, and lanes a–d represent the corresponding autoradiograms. Lanes A and a, intact Dictyostelium myosin; lanes B and b, 0.5-min digestion; lanes C and c, 2-min digestion; lanes D and d, 10-min digestion. HC, heavy chain.

am ide gels such as those in Fig. 3 indicates that the digestion of both phosphorylated and unphosphorylated myosin II is very sensitive to small changes in the ionic strength (Fig. 4). These experiments were performed at a chymotrypsin to myosin ratio of 1:80 (w/w), so that after 2 min at 0 °C only about 80% of the myosin heavy chain was digested in the absence of NaCl. For unphosphorylated myosin II, an increase in the salt concentration to 50 mM is sufficient to decrease by 80% the amount of 38-kDa fragment produced. The digestion of phosphorylated myosin II is affected only slightly by NaCl up to 20 mM, but then is rapidly inhibited as the NaCl concentration increases to 50 mM (Fig. 4). Myosin II in the presence of 0.1 M NaCl can be digested with chymotrypsin if

Fig. 3. Digestion of Dictyostelium myosin II at increasing NaCl concentrations. Unphosphorylated Dictyostelium myosin II at a concentration of 0.2 mg/ml was digested at 0 °C for 2 min at a chymotrypsin to myosin II ratio (w/w) of 1:80. Samples contained 10 mM imidazole, pH 7.5, 1 mM dithiothreitol, and lane A, 0 mM NaCl; lane B, 10 mM NaCl; lane C, 40 mM NaCl; lane D, 100 mM NaCl. Digestions were stopped, and 15-μl samples were electrophoresed on an 8% SDS-polyacrylamide gel as described in the legend to Fig. 1. HC, heavy chain.

Fig. 4. Inhibition of the chymotryptic digestion of Dictyostelium myosin II by increasing ionic strength. Samples of unphosphorylated (●) or phosphorylated (×) myosin II were digested as described in the legend to Fig. 3 at different concentrations of NaCl. Samples of 15 μl were run on 8% SDS-polyacrylamide gels which were then stained with Coomassie Blue. Following destaining, the gels were scanned using an LKB Instruments 2202 Ultrascan laser densitometer. Peaks were cut out and weighed to determine areas. The percent of the heavy chain digested at 0 mM NaCl was determined from the ratio of the areas of the bands representing the intact myosin heavy chain and the 195-kDa fragment. To calculate the percent of the myosin heavy chain digested at other salt concentrations, the area of the 38-kDa fragment band was determined and compared to the area of the 38-kDa band at 0 mM NaCl.

Fig. 5. Solubility of Dictyostelium myosin II at varying NaCl concentrations. Samples (100 μl) of unphosphorylated (●), phosphorylated (×), or chymotrypsin-cleaved (○) myosin II were centrifuged in a Beckman Airfuge at 22 p.s.i. for 15 min at 4 °C. Following centrifugation, the top 80 μl of supernatant was removed and the myosin II concentration determined either by the Bradford assay or, in the case of digested myosin, by Ca2+-ATPase activity. Assays contained 0.19–0.26 mg/ml of myosin in 10 mM imidazole, 1 mM dithiothreitol, pH 7.5, and the appropriate NaCl concentrations. The digest is carried out for longer periods of time or if larger amounts of chymotrypsin are used; however, cleavage no longer occurs exclusively at the 195–38-kDa junction. Multiple sites on the heavy chain are cleaved, and a digestion pattern is observed similar to that obtained by Pagh et al. (16) (data not shown).

There does not seem to be a correlation between the range of ionic strengths over which chymotryptic cleavage at the 195–38-kDa junction is inhibited and the ionic strengths required for myosin II to aggregate into filaments. In the absence of NaCl, unphosphorylated myosin II is soluble (perhaps due to charge repulsion between the myosin tails); however, only 10 mM NaCl is required for nearly all of the myosin to be pelleted as filaments (Fig. 5). Unphosphorylated Dictyostelium myosin II then remains in the form of filaments, as judged by the ability of the myosin to be sedimented, until the salt concentration approaches 0.15 M. Phosphorylated myosin II remains soluble following centrifugation at all of

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the salt conditions tested (Fig. 5), although chymotryptic cleavage of phosphorylated myosin II is strongly inhibited by increasing ionic strength (Fig. 4).

Properties of the Chymotrypsin-cleaved Myosin—Sedimentation studies were also performed on Dictyostelium myosin II following chymotryptic cleavage. At NaCl concentrations between 20 and 80 mM, the majority of the 195-kDa fragment is insoluble and is recovered in the pellet (Figs. 5 and 6). The 38-kDa fragment remains soluble at all salt concentrations, even at those where the 195-kDa fragment pellets (Fig. 6), indicating that the two chymotryptic fragments do not interact strongly with each other. Preliminary results show little difference between the solubility properties of the phosphorylated and unphosphorylated forms of the 38-kDa fragment.

Dictyostelium myosin II that has been digested by chymotrypsin into the 195- and 38-kDa fragments displays a Ca²⁺-ATPase activity of 1.0–1.2 μmol/min/mg, identical to that of intact myosin II, and also retains the ability to bind in an ATP-dependent manner to filaments of actin. Studies carried out in 0.2 M NaCl demonstrate that in the presence of ATP and actin the majority of the 195-kDa fragment is recovered in the supernatant following centrifugation, but in the absence of ATP the 195-kDa fragment is recovered in the actin pellet (data not shown). In all cases, the 38-kDa fragment remains soluble.

Although both the Ca²⁺-ATPase activity and the actin-binding properties of Dictyostelium myosin II are retained following chymotryptic cleavage, the majority of the myosin II actin-activated ATPase activity is lost (Fig. 7). The actin-activated ATPase activity of the chymotrypsin-cleaved myosin remained low even when the actin concentration was increased to 1 mg/ml. After subtracting the Mg²⁺-ATPase activity of intact and digested myosin II in the absence of actin (0.01 μmol/min/mg), the data in Fig. 7 were analyzed using the Hanes-Woolf double-reciprocal plot. Values for V₅₀ of 0.143 and 0.029 μmol/min/mg were obtained for intact and digested myosin II, respectively. Both forms of myosin II had similar Kₐₜₚₐₜ values for actin of 0.24 μM.

Amino Acid Sequence Analysis of the 38-kDa Fragment—The 38-kDa chymotryptic fragment can be purified to near homogeneity in two steps: centrifugation in 50 mM KCl to remove the majority of the 195-kDa fragments (Fig. 6) and chromatography over a Sepharose CL-4B column. The 38-kDa fragment, which elutes from the column with an apparent molecular weight of around 160,000 (data not shown), was subjected to amino acid sequence analysis. A single sequence, extending 28 residues, was obtained (Fig. 8). Boxed residues indicate the repeating pattern of nonpolar amino acids that form the hydrophobic core of the coiled-coil myosin rod.

DISCUSSION

In this paper, conditions are described that restrict the cleavage of Dictyostelium myosin II by chymotrypsin to a small region of the heavy chains, producing two fragments with molecular weights of 195,000 and 38,000 as estimated by SDS-polyacrylamide gel electrophoresis of chymotrypsin-digested myosin before and after centrifugation. Myosin II (0.29 mg/ml) was digested with chymotrypsin at 0 °C for 2 min as described under "Experimental Procedures." KCl was added to 50 mM, and the digest was centrifuged in a Ti-50 rotor at 40,000 rpm for 1 h at 4 °C. Lane A, myosin before digestion; lane B, myosin following digestion; lane C, supernatant after centrifugation; lane D, pellet after centrifugation. Following centrifugation, 10% of the original Ca²⁺-ATPase activity remained in the supernatant, and the rest was recovered in the pellet.

![SDS-PAGE gel](image-url)
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SDS-polyacrylamide gel electrophoresis. Amino acid sequence analysis of the small fragment demonstrates that its amino terminus corresponds to lysine 1826 in the complete sequence of the Dictyostelium myosin II heavy chain (4). If the chymotryptic fragment extends from residue 1826 to the end of the myosin heavy chain (residue 2116), it would consist of 291 amino acids and have a molecular weight, as calculated from the amino acid composition, of 33,700. The elution of this fragment from gel filtration columns with an apparent molecular weight of 160,000 suggests that under native conditions this fragment forms an asymmetric α-helical coiled-coil dimer. The large chymotryptic fragment must then have a molecular weight of close to 209,000 and consist of the globular head regions of myosin II along with the remainder of the tail. This fragment retains both the ATPase and actin-binding sites of the myosin II molecule.

The extreme susceptibility of the peptide bond between residues 1825 and 1826 of the myosin tail to hydrolysis by chymotrypsin must be due in part to the presence of Tyr-1825 in one of the exposed outer positions of the coiled-coil (Fig. 8). The only other two aromatic amino acids in this section of the myosin II sequence, Phe-1795 and Tyr-1816, are both buried within the hydrophobic core of the coiled-coil (4) and so may not be accessible to chymotrypsin. The α-helical coiled-coil structure can be destabilized by the presence of charged or polar residues in hydrophobic core positions (23, 24). A threonine residue occupies the core position immediately preceding Tyr-1825 while, just after Tyr-1825, glutamic acid, lysine, and threonine residues fill the three consecutive core positions (Fig. 8). However, charged and polar residues occur relatively frequently in core positions throughout the Dictyostelium myosin II tail (4), and a more extensive analysis is required before it can be concluded that the section of the coiled-coil around Tyr-1825 is significantly less stable than other regions of the tail.

Chymotryptic digestion at Tyr-1825 is strongly inhibited as the salt concentration is increased from 10 to 60 mM (Figs. 3 and 4). Over this range of ionic strengths, unphosphorylated myosin II remains filamentous. The inhibition of digestion could therefore be due to some alteration in the packing of myosin II molecules within the bipolar filament, resulting in the region of the tail around Tyr-1825 become inaccessible to chymotrypsin. However, chymotryptic digestion of unphosphorylated myosin II remains inhibited even at 0.2 M NaCl, a salt concentration at which unphosphorylated myosin II is monomeric. In addition, chymotryptic digestion of phosphorylated myosin II is inhibited by increasing salt concentrations, although the phosphorylated myosin is soluble at all ionic strengths. Therefore, the inhibition of chymotryptic digestion is not dependent on the presence of filaments of myosin II, but is probably due to a local conformational change in the region of the myosin tail around Tyr-1825 as the ionic strength increases. Further studies will be required to determine whether this local conformational change is reflected in the structure of myosin II filaments or perhaps in an alteration in actin-activated ATPase activity.

Conformational changes near the end of the tail of Dictyostelium myosin II are clearly of physiological importance, because phosphorylation within this region (Fig. 2) results in a dramatic decrease in the ability of the myosin to form filaments (Fig. 4). Whereas greater than 90% of unphosphorylated myosin II sedimented at NaCl concentrations of 10-100 mM, less than 20% of the phosphorylated myosin II (1 mol of phosphate/mol of heavy chain) sedimented. Earlier studies indicated that about 50% of phosphorylated Dictyostelium myosin II sedimented at these salt concentrations (7); however, the myosin II used in these studies contained only 0.6 mol of phosphate/mol of heavy chain, with an unknown amount of the phosphate present on serine residues. Along with previous results (15), the studies in this paper demonstrate that phosphorylation of a threonine residue near the end of the tail of Dictyostelium myosin II with the 700-kDa Dictyostelium heavy chain kinase is sufficient both to inhibit the actin-activated ATPase activity of myosin II and its ability to polymerize.

In addition to the 700-kDa kinase, which phosphorylates only threonine residues, a lower molecular weight Dictyostelium kinase that phosphorylates both serine and threonine residues within the tail region of myosin II has been identified (13). Also, Maruta et al. (14) have isolated two threonine-specific kinases that elute from gel filtration columns with molecular weights of 70,000. The threonine(s) phosphorylated by one of the 70-kDa kinases has been located, by using monoclonal antibodies specific for different sections of the Dictyostelium myosin II molecule, to a region of the myosin tail about 32 kDa from the carboxyl terminus of the myosin (16).

Thus, despite considerable differences in their properties, all of the Dictyostelium myosin II heavy chain kinases so far studied phosphorylate sites on the myosin II tail, whereas both the high and low molecular weight threonine-specific kinases are now known to phosphorylate sites near the end of the tail. The chymotryptic digest described in this paper provides a simple means by which to obtain large amounts of the phosphorylated tail-end fragment of Dictyostelium myosin II. Further proteolysis of this fragment should allow the eventual identification of the phosphorylated amino acids within the myosin II heavy chain sequence. It will be important to determine whether the same threonine residue is phosphorylated by each of the Dictyostelium myosin II heavy chain kinases so far isolated (in which case the possibility must be considered that the kinases represent different forms of a single enzyme) or whether each kinase phosphorylates a different site on the myosin II heavy chain, perhaps with different effects on the contractile activity of the myosin.

Two other conventional myosins, from Acanthamoeba (25, 26) and rabbit macrophages (27), have previously been shown to contain heavy chain phosphorylation sites located very close to the carboxyl-terminal tail end of the molecule. In both cases, this regulatory region could be specifically removed by proteolytic digestion. Acanthamoeba myosin II lacking the regulatory region displays neither actin-activated ATPase activity nor the ability to form filaments (28). In contrast, removal of the tail end of Dictyostelium myosin II does not prevent the formation of filaments, although the filaments formed by the chymotrypsin-cleaved myosin are less stable than filaments formed by intact unphosphorylated myosin II (Fig. 4). These results are in good agreement with studies employing monoclonal antibodies which have shown that two sections of the Dictyostelium myosin II tail are important for filament formation (29, 30). Monopolar and bipolar filament formation is completely inhibited by antibodies that bind to the tail 50-80% of the distance from the heads, whereas bipolar (but not monopolar) filament assembly is abolished by an antibody that binds to the tip of the tail (30). It is surprising that the carboxyl-terminal end of the tail is not absolutely required for filament formation, yet it contains a site which when phosphorylated is able to prevent filament formation by the whole myosin II molecule.

The $V_{max}$ for the actin-activated ATPase of Dictyostelium myosin II is inhibited 80% by the removal of the tail end of
the molecule (Fig. 7). Because the filament-forming assays and ATPase assays are performed under different conditions, we cannot be certain that the chymotrypsin-cleaved myosin is filamentous in the ATPase assay. However, it has been shown that a soluble S-1 fragment prepared from *Dictyostelium* myosin II has a *K*ₐₐₚₑₚₑ only 12 times greater than that of filamentous myosin (17). Therefore, even if the chymotrypsin-cleaved myosin were soluble in the ATPase assay, the actin concentrations we have tested (up to 1 mg/ml actin) should have been sufficient to reach *V*ₘₐₓ. The results suggest that inhibition of actin activation is not due solely to the weakened ability of the digested myosin to form filaments.

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