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Cytochalasin B, Its Interaction with Actin and Actomyosin from Muscle

(cell movement/microfilaments/rabbit striated muscle)

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ABSTRACT **Cytochalasin B, an alkaloid that inhibits a wide variety of cellular movements, interacts with actomyosin, the contractile protein complex of striated muscle. This interaction causes a decrease in viscosity of the actomyosin complex and an inhibition of acto-heavy meromyosin ATPase activity of at least 60%. Cytochalasin B does not affect the viscosity of myosin nor the ATPase activity of heavy meromyosin, suggesting that the drug might interact directly with the actin moiety of the actomyosin complex. Indeed, as judged by viscometry, there is a strong interaction of cytochalasin B with actin, at nearly stoichiometric concentrations. Myosin appears to compete with cytochalasin for binding to actin.**

All living cells show movement. The movement may take many forms, such as intracellular motility of cell organelles and cytoplasm, amoeboid movement, and other alterations in cell shape apparently caused by contractile events within cells. Most types of cellular movement may be related at the molecular level. Many investigators have suggested that movements in general are mediated by contractile proteins similar to actomyosin from striated muscle (for review, see ref. 1). Indeed, actin-like proteins, and to a lesser extent myosin-like proteins, have been identified in extracts of many cells other than muscle (1), including blood platelets (2, 3), leucocytes (4), mammalian brain (5), sarcoma cells (6), sea-urchin eggs (7), *Acanthamoeba castellanii* (8), plasmodia of the acellular slime mold, *Physarum polycephalum* (9–11), amoebae of the cellular slime mold, *Dictyostelium discoideum* (12), and most recently in epithelial cells of the chicken intestine (13). The similarity of these actin-like proteins with muscle actin is emphasized by the observations (11–16) that they interact with muscle heavy meromyosin (HMM) to give "arrowheads" similar to those originally observed by Huxley with muscle acto-HMM (17).

Another indication that the molecular basis of many forms of movement may be related comes from studies using the drug cytochalasin B. This alkaloid, a metabolite of the fungus *Helminthosporium dematioideum* (18), inhibits a wide variety of cellular movements (18–20). To our knowledge, nothing is known about the mode of action of cytochalasin B at the molecular level.

Since actomyosin-like proteins and cytochalasin B have both been implicated in cellular movement in several organisms, we have begun to investigate whether cytochalasin B inhibits movement by interacting with actomyosin-like proteins. We report here that cytochalasin B interacts with purified muscle actin and that it appears to compete with myosin for binding to the actin.

Abbreviations: Actin, filamentous actin or F-actin (the monomer is specified as G-actin); CB-actin, actin saturated with cytochalasin B; Me_2SO, dimethyl sulfoxide; HMM, heavy meromyosin (a proteolytic fragment of myosin retaining ATPase activity and the ability to interact with actin).

MATERIALS AND METHODS

Reagents. Cytochalasin B (molecular weight = 479; see ref. 21) was obtained from Imperial Chemical Industries Ltd., Cheshire, England. It was stored at 4°C as a 10 mg/ml stock solution in dimethyl sulfoxide (Me_2SO).

Protein Preparations. Actin was purified to electrophoretic homogeneity from an acetone powder of striated muscle from rabbit according to the 0.6 M KCl purification procedure of Spudich and Watt (22). Myosin was prepared from rabbit striated-muscle, as described by Tonomura *et al.* (23), and HMM was obtained by cleavage of myosin with trypsin, followed by precipitation of light meromyosin and of the remaining myosin at low ionic strength (24).

Assay of Adenosine Triphosphatase Activity. The ATPase activity of acto-HMM (in the presence of 0.2 mM Ca^{2+}) was determined as described (22), except for minor modifications described in the legends.

Viscometry. The viscosity of the various protein solutions was determined with a conventional Ostwald viscometer, with a flow time for water of about 100 sec. The temperature was held constant at 25°C with a water bath. The reduced viscosity, η_{red}, is $(\eta_{rel} - 1)/c$, where η_{rel} is the flow time for the protein solution divided by the flow time for the corresponding buffer, and c is the protein concentration in mg/ml.

Other Methods. Protein concentration was determined after acid precipitation by the method of Lowry *et al.* (25).

RESULTS

Cytochalasin B interacts with actomyosin and acto-HMM

Cytochalasin B Causes a Decrease in the Viscosity of Actomyosin. The effect of cytochalasin on the viscosity of actomyosin was shown in two experiments. In the first, the actomyosin complex was formed before the addition of cytochalasin; in the second, the actomyosin complex was formed after the addition of cytochalasin.

The results of the first experiment were as follows (Fig. 1, *open circles*). Addition of actin to myosin in a 5:1 molar ratio caused an increase in the viscosity of the solution to a value

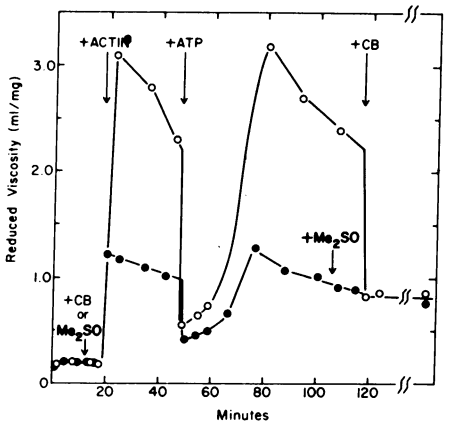

FIG. 1. Effect of cytochalasin B on the viscosity of actomyosin. *Arrows:* at 12 min, 0.05 ml of Me_2SO (O) or 0.05 ml of a 10 mg/ml solution of cytochalasin B (CB) in Me_2SO (●) was added to 3.1 ml of a myosin solution [0.81 mg myosin/ml–16 mM potassium phosphate buffer (pH 7)–0.5 M KCl]. At 19 min, 0.3 ml of F-actin (2.0 mg) was added to the Me_2SO-treated myosin (O) and to the cytochalasin-treated myosin (●). At 48 min, 0.1 ml of 35 mM ATP (pH 7) was added to the Me_2SO-treated actomyosin (O) and to the cytochalasin-treated actomyosin (●). At 106 min, 0.05 ml of Me_2SO was added to the cytochalasin-treated actomyosin (●), and at 117 min, 0.05 ml of a 10 mg/ml solution of cytochalasin B was added to the Me_2SO-treated actomyosin (O). The points to the right of the break (⟆) in the curve denote viscosity determinations more than 2 hr later.

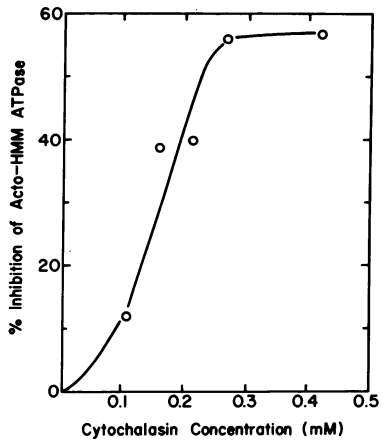

FIG. 2. Inhibition of acto-HMM ATPase by cytochalasin B. Percentage inhibition was determined by comparison of the acto-HMM ATPase activity in the presence of cytochalasin in Me_2SO to that in the presence of Me_2SO alone. The activity of HMM alone was subtracted from the total ATPase activity to yield the actin-activated activity (20–60 μmol P_i per hr per mg of HMM per mg of actin). The Me_2SO did not have a significant effect on the acto-HMM ATPase activity. Some variability was observed in the assays with Me_2SO and cytochalasin. Thus, the above points represent averages of up to five determinations (which differed from one another by as much as 25% inhibition) at a given cytochalasin concentration, where each determination was an average of at least duplicate assays. Maximum inhibition was observed at about pH 7.4, with final concentrations of Tris and maleate of 12.5 mM each; otherwise, ATPase activity was determined as described (22).

(3.1 ml/mg) far above the viscosities for myosin* or actin alone (about 0.2 ml/mg and 0.8 ml/mg, respectively). This highly viscous actomyosin complex was rapidly dissociated by the addition of ATP, but the actin and myosin reassociated within 30 min (as the ATP was hydrolyzed). These viscosity changes are well-known characteristics of muscle actomyosin (26). Addition of cytochalasin at 117 min caused a large and rapid decrease in the viscosity of the actomyosin complex.

The results of the second experiment, in which the actomyosin complex was formed after the addition of cytochalasin, were as follows (Fig. 1, *filled circles*). The viscosity of myosin alone was not affected by addition of up to 0.3 mM cytochalasin. Addition of actin to the mixture of myosin and cytochalasin in 0.5 M KCl resulted in the formation of an actomyosin complex that had a viscosity much lower than that obtained in the absence of cytochalasin. Cytochalasin did not prevent the dissociation of actomyosin by ATP, and the actin and myosin reassociated within 30 min as before (thus, the time required for the myosin to hydrolyze the ATP was not affected by the presence of cytochalasin. It is important to note that in 0.5 M KCl, actin-activation of myosin ATPase does not occur and all of the ATPase activity is from myosin alone). The final viscosity of the actomyosin in this experiment was the same as that obtained by adding cytochalasin to the preformed actomyosin complex.

Cytochalasin B Inhibits Actin-Activation of HMM ATPase. It was important to determine whether the interaction of cytochalasin B with actomyosin results in inhibition of the actin-activated myosin ATPase activity, since this activity is believed to be intimately related to movement during muscle contraction. We used HMM for this study since HMM, unlike myosin, is soluble under the conditions of our ATPase assay. We found that the actin-activation of HMM ATPase activity was inhibited up to about 60% by the addition of cytochalasin B (Fig. 2). A precipitate was apparent in the ATPase reaction mixtures containing 0.42 mM cytochalasin B, indicating that the cytochalasin was not completely soluble at this concentration.

Cytochalasin B does not appear to interact with myosin

The above experiments demonstrate that cytochalasin interacts with actomyosin. Does cytochalasin interact with myosin, with actin, or with both? It was already apparent that the viscosity of myosin alone was not affected by the drug (see Fig. 1). We also found that the ATPase activity of HMM alone (HMM is equivalent to myosin, in that its ATPase activity is activated by actin) was not significantly inhibited by cytochalasin†. This finding is consistent with our observation that the time required for myosin to hydrolyze ATP, as judged by viscosity measurements, was not affected

* These experiments were performed under conditions (0.5 M KCl) where myosin exists in its depolymerized form.

† Three independent determinations assayed as described (22) gave 1.93 ± 0.16 μmol of P_i per hr per mg of HMM in Me_2SO and 1.94 ± 0.12 μmol of P_i per hr per mg of HMM in concentrations of cytochalasin B ranging from 0.20 to 0.60 mM (0% inhibition). Two independent determinations assayed with the modifications described in the legend to Fig. 2 gave 0.89 ± 0.07 μmol of P_i per hr per mg of HMM in Me_2SO and 0.80 ± 0.02 μmol of P_i per hr per mg of HMM in concentrations of cytochalasin B ranging from 0.20 to 0.25 mM (10% inhibition).

by cytochalasin (see Fig. 1). Thus, we have no evidence indicating an interaction of cytochalasin with myosin; therefore, we examined its effect on actin alone.

Cytochalasin B interacts with actin

Cytochalasin B Causes a Decrease in the Viscosity of Actin. It is well known that G-actin polymerizes in the presence of 0.1 M KCl to a filamentous form, F-actin. This polymerization, in the absence of cytochalasin, resulted in an increase in viscosity, to a value of about 0.8 ml/mg (Fig. 3, *open circles*). Polymerization of G-actin in 0.1 M KCl occurred in the presence of cytochalasin B, but the viscosity of the resultant polymerized actin (which we have termed CB-actin) was only 0.5 ml/mg (Fig. 3, *filled circles*). Addition of cytochalasin B to formerly polymerized actin rapidly transformed the F-actin to the CB-actin form (Fig. 3, *half-open circles*). It appears, therefore, that CB-actin can be formed by polymerization of G-actin in the presence of cytochalasin or by addition of cytochalasin directly to F-actin.

The reduced viscosity of F-actin increases with actin concentration, since the interaction of actin filaments with one another becomes greater at higher concentrations of actin. To determine whether the intrinsic viscosity (the reduced viscosity extrapolated to zero protein concentration) of F-actin is affected by cytochalasin B, the reduced viscosity at various actin concentrations was measured in the presence of five different concentrations of cytochalasin. The intrinsic viscosity is shown as a function of cytochalasin B concentration in Fig. 4. The decrease in intrinsic viscosity from the F-actin form (0.7 ml/mg) to the CB-actin form (0.3 ml/mg) was more than 70% with 0.05 mM cytochalasin B. This concentration of cytochalasin is similar to the concentrations of actin used in the experiments of Fig. 4, indicating that the maximal effect on the viscosity of actin occurs with nearly stoichiometric concentrations of cytochalasin B and actin.

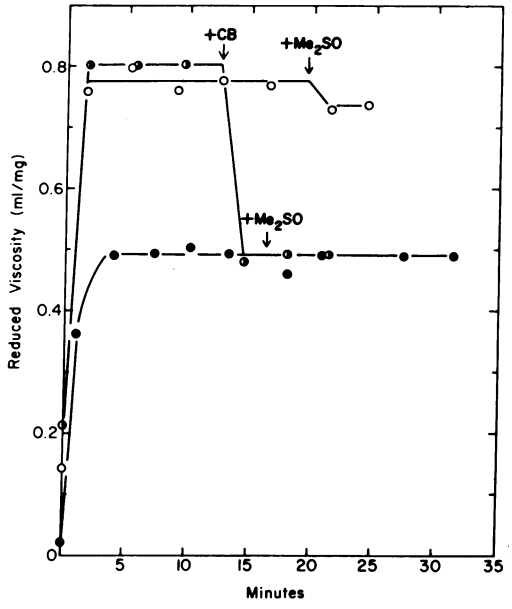

FIG. 3. Effect of cytochalasin on the polymerization of G-actin and on the viscosity of F-actin. G-actin (2.9 ml, 1.1 mg/ml, η_{red} = 0.05 ml/mg) in Buffer A (see Table 1) was mixed with 50 μl of Me_2SO (○,◑) or with 50 μl of a 10 mg/ml solution of cytochalasin B (●). Me_2SO alone, but not Me_2SO containing cytochalasin, routinely caused a slow increase (over the period of about 1 hr) in the reduced viscosity of the G-actin solution (to a value near 0.2 ml/mg). About 45 min after the addition of Me_2SO, 0.10 ml of 3 M KCl was added; this was called *time zero*. 0.5 mg of cytochalasin B (CB, *arrow*) was added to F-actin at 12.5 min (◑). 50 μl of Me_2SO was added (*arrows*) to CB-actin (●) and F-actin (○) at 16.5 and 19.5 min, respectively, as controls.

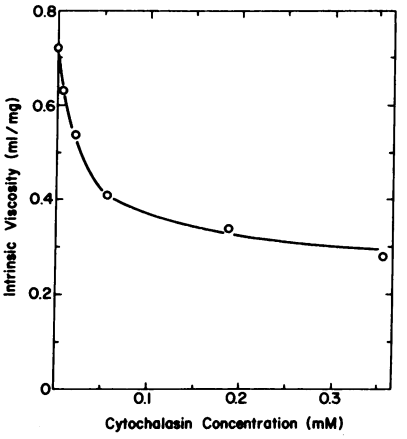

FIG. 4. Intrinsic viscosity of F-actin as a function of cytochalasin B concentration. Reduced viscosities of F-actin in the presence of various concentrations of cytochalasin B were measured at concentrations of actin ranging from 0.007 to 0.028 mM. Extrapolation of the reduced viscosity to zero actin concentration yielded the intrinsic viscosity at each concentration of cytochalasin B.

Myosin Appears to Compete‡ with Cytochalasin B for Binding to Actin. One experiment that suggests competitive binding of myosin and cytochalasin is shown in Fig. 5. Addition of F-actin to myosin in a 1:2 molar ratio increased the viscosity of the solution to 0.42 ml/mg. Addition of CB-actin to myosin also increased the viscosity of the solution, but only to 0.33 ml/mg. The important observation is that addition of cytochalasin to the preformed actomyosin complex resulted in a very slow decrease in viscosity toward 0.33 ml/mg. This slow decrease in viscosity is in contrast to the very rapid effect of cytochalasin on the viscosity of F-actin (see Fig. 3), and on that of actomyosin formed with an excess of actin (see Fig. 1). Thus, myosin appears to interfere with the interaction of cytochalasin and actin.

A further observation that is consistent with competition between myosin and cytochalasin for binding to actin is that the concentration of cytochalasin needed for 60% inhibition of acto-HMM ATPase activity (about 0.25 mM cytochalasin, see Fig. 2) is nearly ten times that required for 60% of the maximal decrease in intrinsic viscosity of actin alone (about 0.03 mM cytochalasin, see Fig. 4).

Yet another indication that myosin and cytochalasin compete for binding to actin is the observation that myosin appears to displace cytochalasin from CB-actin, as suggested by the following viscosity experiment (Table 1). Addition of myosin to undialyzed CB-actin (final concentration of cytochalasin was 0.057 mM) increased the viscosity of the solution to 1.74 ml/mg. On the other hand, addition of myosin to

‡ The term "compete" is used here to refer to mutual inhibition of binding to actin without specifying the type of competition (e.g., competitive, noncompetitive, or allosteric).

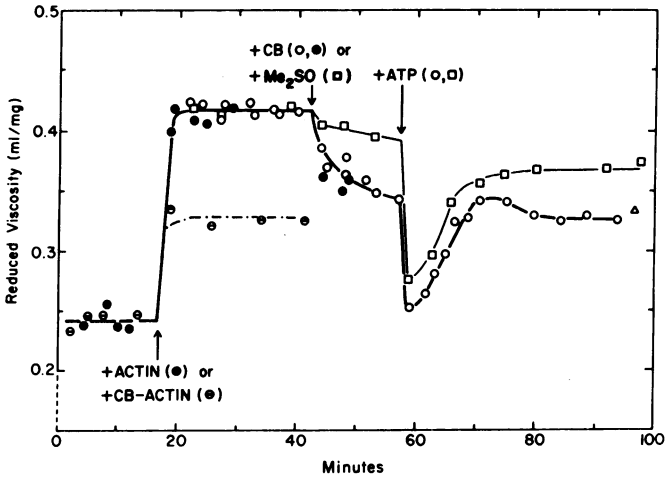

FIG. 5. Effect of cytochalasin B on the viscosity of an actomyosin complex formed with a molar excess of myosin. At 16.5 min (*arrow*), 0.25 ml of a 1.8 mg/ml solution of F-actin (●) or of CB-actin (⊖; prepared by prior incubation of F-actin in 1.7 mg/ml of cytochalasin B) was added to 2.9 ml of a myosin solution [2.45 mg myosin/ml–15 mM potassium phosphate buffer (pH 7)–0.5 M KCl]. At 42 min (*arrow*), 50 μl of a 10 mg/ml solution of cytochalasin B (CB) was added to the actomyosin (●). In another series of experiments that began with preformed actomyosin (○,□; actin and myosin concentrations were the same as described above, but 25 μl of Me_2SO was present), at 42 min (*arrow*), 25 μl of Me_2SO (□) or 25 μl of a 10 mg/ml solution of cytochalasin B (○) was added to the actomyosin. At 57 min (*arrow*), 0.1 ml of 35 mM ATP (pH 7) was added to the Me_2SO-treated actomyosin (□) and to the cytochalasin-treated actomyosin (○). In one experiment (△, *see 97 min*) no ATP was added after the cytochalasin addition at 42 min; otherwise, the conditions were as described (○).

CB-actin that was dialyzed§ to reduce the concentration of free cytochalasin (final concentration of cytochalasin would be only 0.003 mM if 1 mole of actin binds about 1 mole of cytochalasin) increased the viscosity of the solution to 2.22 ml/mg. The difference in viscosity observed upon addition of myosin to the CB-actin before and after dialysis would be explained if myosin displaces cytochalasin from CB-actin and competes with free cytochalasin for binding to actin.

If myosin (or HMM) can indeed displace cytochalasin from CB-actin, then actin treated with an excess of cytochalasin and then diluted to reduce the concentration of cytochalasin to 0.03 mM should activate the HMM ATPase normally. CB-actin formed by the addition of cytochalasin B (to a final concentration of 0.3 mM) to F-actin, CB-actin formed by the polymerization of G-actin in the presence of 0.3 mM cytochalasin, and Me_2SO-treated actin (all from the experiment illustrated in Fig. 3) were diluted 1:10 into ATPase reaction mixtures so that, in the case of the CB-actin, the final concentration of cytochalasin was 0.03 mM. There was no difference in acto-HMM ATPase activity among the three actin preparations (18.2 ± 0.7 μmol of P_i per hr per mg of HMM per mg of actin).

The above results suggest that myosin and cytochalasin may compete with one another for binding to actin, but further experiments are necessary to establish the detailed nature of these interactions.

§ The viscosity of CB-actin was not altered by extensive dialysis against buffer containing no cytochalasin (Table 1).

DISCUSSION

Wessells *et al.*, using a crude preparation of actomyosin from mouse striated-muscle, reported (20) that 0.1 mM cytochalasin B did not inhibit the ability of the actomyosin to form a heavy precipitate in the presence of ATP (a phenomenon called "superprecipitation"). We have shown, using actomyosin reconstituted from purified actin and myosin from rabbit striated-muscle, that there is in fact an inhibitory effect of cytochalasin B on the actomyosin. In view of our results, we believe that an interaction of cytochalasin with actin or actin-like proteins *in vivo* could account for the ability of cytochalasin to inhibit various forms of cell motility and contraction.

In striated-muscle, actin is the major component of the 70 Å-diameter "thin filaments" that are part of the highly organized sarcomere. What is the form of the actin-like proteins in other types of cells? Recently, attention has been focused on a class of cellular filaments, called "microfilaments", that have about the same diameter as does actin. The locations of microfilaments within cells, as determined by electron microscopy, suggest that they may be involved in cytoplasmic streaming, cytokinesis, nerve axon production, changes in cell shape during development of an embryo, blood-clot retraction, amoeboid movement, and other forms of cell motility (for reviews, see refs. 1 and 20). Further evidence that microfilaments may be involved in cell movement was provided by the electron microscopic studies of Schroeder (19) and Wessells *et al.* (20) that indicated that the structure of microfilaments within cells is reversibly disrupted by cytochalasin B. Inasmuch as our results show that cytochalasin does interact with actin, and since other investigators have shown that microfilaments and actin have similar dimensions, it is tempting to speculate that microfilaments may indeed be actin-like proteins, although this identity remains to be determined.

TABLE 1. *Effect of dialysis and addition of myosin on the viscosity of actin and of CB-actin*

Sample	Reduced viscosity (ml/mg)	
	Before dialysis	After dialysis
Actin	0.84	0.84
CB-actin	0.61	0.61
Actomyosin	3.05	3.05
CB-actomyosin	1.74	2.22

F-actin (3.0 ml, 1.2 mg/ml in Buffer A [5 mM Tris·HCl (pH 8)–0.2 mM ATP–0.5 mM 2-mercaptoethanol–0.2 mM $CaCl_2$] containing 0.1 M KCl) was mixed with 25 μl of cytochalasin B solution (10 mg/ml) or with 25 μl of Me_2SO. After the viscosity of the two samples was determined, they were dialyzed for 18 hr against 300 volumes of Buffer A containing Me_2SO (0.8%), with a change of buffer after the first 3 hr; under these conditions, F-actin depolymerizes to G-actin. The dialyzed actin in each case was then repolymerized by the addition of 3 M KCl, to a final concentration of 0.1 M, and the viscosity of the solutions was determined. The actomyosin solutions were prepared by mixture of 1-ml aliquots of the above actin solutions with 2 ml of myosin [0.8 mg/ml in 0.5 M KCl–0.02 M potassium phosphate buffer (pH 7.0)]. The viscosity of the solutions was determined after 20 min.

What is the alteration in F-actin structure upon the addition of cytochalasin B? The observed decrease in viscosity could be a result of a change in the axial ratio of the F-actin filaments, either by partial depolymerization or by an increase in width of the filaments, or it could be a result of a decreased rigidity of the actin "rod".

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