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alveolar macrophages
kinase
myosin

PMN myosin light chain
polymorphonuclear leukocytes

Purification of Myosin Light Chain Kinase from Rabbit Polymorphonuclear Leukocytes

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Summary

To investigate the regulation of actin-myosin interaction in rabbit phagocytic cells, purified myosin and a partially purified cofactor protein were obtained from polymorphonuclear leukocytes (PMN) and alveolar macrophages (ALM) by molecular sieve filtration over an agarose column. ALM cofactor enhanced the Mg^{++}-ATPase activity of ALM myosin by actin to 0.15 ± 0.04 μmoles P_i per mg myosin per min and PMN cofactor enhanced PMN myosin to 0.43 ± 0.03 μmoles P_i per mg myosin per min. The crude cofactor preparations isolated from the two types of leukocyte extracts were interchangeable with the leukocyte myosins. When ALM cofactor was added to a PMN actomyosin complex, the Mg^{++}-ATPase activity of the PMN myosin was 3-fold higher than with ALM cofactor and its own actomyosin complex. In contrast, PMN cofactor did not enhance ALM actomyosin Mg^{++}-ATPase activity beyond that observed with ALM cofactor and ALM actomyosin. Cofactor protein from the PMN was further purified on a DEAE-Sephagel column. After electrophoresis on sodium dodecyl sulfate-polyacrylamide gel, the isolated fraction weighed 70,000 daltons. This fraction stimulated the actin-mediated myosin Mg^{++}-ATPase. In the presence of Mg^{++} and [γ^{32}P]ATP, the 70,000 dalton protein phosphorylated the 20,000 dalton light chain of PMN myosin, leading to the incorporation of 0.62 ± 0.09 moles of P_i per mole myosin. On the basis of these results, we propose that phagocytic cofactor is a kinase which regulates the enzymatic activity of phagocytic cell myosin.

Speculation

The identification of actin, myosin, and related protein in eukaryotic cells raises questions as to the mechanisms involved in the control of these proteins. The results obtained in this study indicate that phosphorylation of the 20,000 daltons light chain may have a crucial role in regulating actin-myosin interaction in phagocytic cells.

Myosin has been isolated from a wide variety of nonmuscle cells including guinea pig and human polymorphonuclear leukocytes (PMN) and rabbit alveolar macrophages (ALM) (3, 10, 21, 22). These myosins are composed of two heavy chains of 200,000 daltons and four light chains, two each of 20,000 and 16,000 daltons. Unlike skeletal muscle myosin, the nonmuscle myosins have uniformly exhibited low Mg^{++}-ATPase activities even in the presence of amounts of F-actin that markedly stimulate the Mg^{++}-ATPase activity of skeletal muscle myosin (11). It is now known that actin activation of nonmuscle myosin Mg^{++}-ATPase requires other proteins. One such discovery was the cofactor protein of *Acanthamoeba castellanii*, that is required for actin activation of *acanthamoeba* myosin I (17). The cofactor protein of *Acanthamoeba* is a kinase that specifically catalyzes the phosphorylation of the 140,000-dalton heavy chain of *Acanthamoeba* myosin I (14). A similar cofactor protein was observed in ALM, but the function of macrophage cofactor protein has remained unknown (21). In instances of other nonmuscle cells, actin activation of the Mg^{++}-ATPase activity occurs only after phosphorylation of the 20,000-dalton light chain by specific kinases (1, 5, 8, 26).

In this paper, we report the isolation and purification from rabbit PMN of a cofactor protein shown to a protein kinase which is responsible for phosphorylating the PMN myosin light chain. We also report on the interchangeable activity of crude cofactor protein isolate from PMN and ALM with the respective leukocyte myosins.

EXPERIMENTAL PROCEDURE

MATERIALS AND METHODS

Adenosine 5′-triphosphate (ATP), dithiothrietol (DTT), Tris-Base, Tris-HCl, and Tris-maleate were purchased from Sigma Chemical Company (St. Louis, MO). Agarose, sodium dodecyl sulfate, acrylamide, and bis-acrylamide were purchased from Bio-Rad Laboratories (Richmond, CA). Ammonia persulfate was purchased from Fischer Scientific Company (Cincinnati, OH), and ultrapure urea was purchased from Schwartz-Mann (Orangeburg, NY). DEAE-Sephacel was obtained from Pharmacia Fine Chemicals Company (Piscataway, NJ). Other chemicals were reagent grade, and deionized water was used throughout the studies.

CELL PREPARATION

PMN. Three to eight white New Zealand albino rabbits received intraperitoneal injections of 100 ml of sterile 12% caseinate in 0.15 M NaCl (20). Sixteen to 20 hr later, the animals were sacrificed, and exudate cells were washed out of the peritoneal cavities with 0.15 M NaCl at 25°C and suspended in 0.34 M sucrose, 20 mM Tris-HCl, pH 7.0, 10 mM DTT, and 0.5 mM ATP (22). The cells were disrupted in a chilled Dounce homogenizer with a tight-fitting pestle. Homogenates were prepared according to the method of Stossel and Pollard (22) with the addition of 2 units aprotinin per ml of homogenate.

ALM. Three to eight New Zealand albino rabbits received intravenous injections of sterile complete Freud's adjuvant in the ear vein, which induces large quantities of macrophages to collect in the lungs (15). Two to 3 wk later, the animals were anesthetized with sodium pentobarbitol, and the macrophages were washed out of the lungs by intratracheal lavage with 0.15 M NaCl at 25°C. The cells were treated thereafter by the method of Stossel and Hartwig (21) for isolation of contractile protein. Wright-stained smear of the PMN and ALM preparaion to the 95% mature PMN and ALM, respectively. Platelets and erythrocytes were not present.

PURIFICATION OF PHAGOCYTIC CELL MYOSIN AND COFACTOR

Protein was determined by the method of Lowry *et al.* (13) using bovine serum albumin as a standard. Rabbit F-actin was purified from the back and leg muscles of rabbits according to the

method of Spudich and Watt (19). Myosin and cofactor protein were isolated from PMN and ALM by the method of Stossel and Hartwig (21) with a few modifications. Purification of myosin was carried out by filtration on an agarose column (1.5 x 30 cm) of Bio-Gel A-15 m (200 to 400 mesh) previously equilibrated with 0.5 M KCl in 20 mM Tris-HCl, pH 7.2-1 mM EDTA-2.5 mM DTT and eluted with the same buffer. The fractions of myosin with its K^+-EDTA ATPase activity were pooled and dialyzed at 4°C for 16 to 20 hr against 20 mM Tris-HCl, pH 7.2, containing 0.5 M KCl, 0.2 mM DDT, and 0.1 mM EDTA; the solution was centrifuged at 19,000 × *g* for 30 min to remove insoluble material. The supernatant containing myosin was concentrated to 0.5 mg to 1 mg/ml, and the ionic strength was lowered to 30 mM KCl by dialysis against 20 mM Tris-HCl, pH 7.2-30 mM KCl-1 mM EDTA-0.2 mM DTT for determination of enzymatic activity.

FURTHER PURIFICATION OF COFACTORS ON DEAE-SEPHACEL CHROMATOGRAPHY

The second protein peak (Fig. 1, *bars*) obtained from the Bio-Gel A-15 m column and containing cofactor activity was pooled and centrifuged at 100,000 × *g* at 4°C for 3 hr. The supernatant was concentrated and dialyzed against the starting buffer (20 mM Tris-HCl, pH 8.0-1 mM DTT-0.34 M sucrose) before applying to the column (2.5 x 10 cm) of DEAE-Sephacel and eluted with a linear gradient of KCl. The effluents were collected and measured by absorption at 280 nm in a Gilson Recording UV Column Monitor (Gilson Medical Electronics, Inc.). The purified cofactor activity obtained from the ion-exchange column of DEAE-Sephacel was assayed, and the fractions were pooled and dialyzed against the starting buffer containing 50 mM KCl and then concentrated in dialysis sac by polyethylene glycol and stored at −20°C until used.

ASSAY OF MYOSIN ATPase ACTIVITY

Assays were performed at 37°C in 1.0 ml of 0.6 M KCl-1.1 mM ATP, 20 mM Tris-maleate buffer solution, pH 7.0 with either 2 mM EDTA, 5 mM $CaCl_2$, or 5 mM $MgCl_2$ present for determination of K^+-EDTA activated, K^+-Ca^{++} activated and K^+-Mg^{++}-ATPase activity, respectively (22). The amount of inorganic phosphate released during the reaction was determined by a previously described method (3). The conditions for the measurement of Mg^{++}-dependent actin activation ATPase activity were 20 mM KCl-1.0 mM ATP-2 mM $MgCl_2$-20 mM Tris-maleate buffer, pH 7.0, at 37°C. For determination of cofactor (kinase) activity, the reaction mixture consisted of 0.1 mg/ml F-actin, 0.015 mg/ml or 0.02 mg/ml myosin and 0.1 ml of column fraction as described in the respective legends. The crude and purified cofactor activities are expressed by the increase in specific activity of myosin ATPase as μmoles P_i/min/mg myosin protein.

GEL ELECTROPHORESIS

Samples of PMN, ALM myosin, and PMN myosin kinase (purified cofactor) were denatured with sample buffer (0.01 M sodium phosphate buffer, pH 7.0, containing 1% sodium dodecyl sulfate (SDS) and 1% mercaptoethanol) in boiling water for 5 min and applied to cylindrical 7.5% acrylamide gels (5 x 75 mm) containing 0.1% SDS according to the method of Weber *et al.* (24). Standard proteins of known molecular weight (Bio-Rad Laboratories), were used to calibrate the gel columns for determining the molecular weight of unknown proteins (7).

PHOSPHORYLATION ASSAY

Phosphorylation of PMN myosin was carried out at 37°C using a procedure similar to that described by Scordilis and Adelstein (18), and Hesketh *et al.* (9). The reaction mixture (total volume = 1.0 ml) contained 0.1 to 0.3 mg of purified myosin, 0.05 to 0.1 mg purified kinase in 20 mM Tris-HCl, pH 7.2-10 mM $MgCl_2$-0.2 M KCl with the addition of stock ATP solution (Premix 1 ml of 0.5 mM ATP plus 80 μCi of [γ-^{32}P]ATP; specific activity, 3.0 Ci/mmole) to a final concentration of 50 μM. For analysis of trichloroacetic acid precipitable ^{32}P incorporation, the reaction was stopped after 30 min incubation by the addition of 1 ml of 20% trichloroacetic acid, 4% sodium pyrophosphate, and bovine serum albumin (0.1 mg/0.1 ml) as a carrier. The precipitate was collected by centrifugation and washed thoroughly with several volumes of

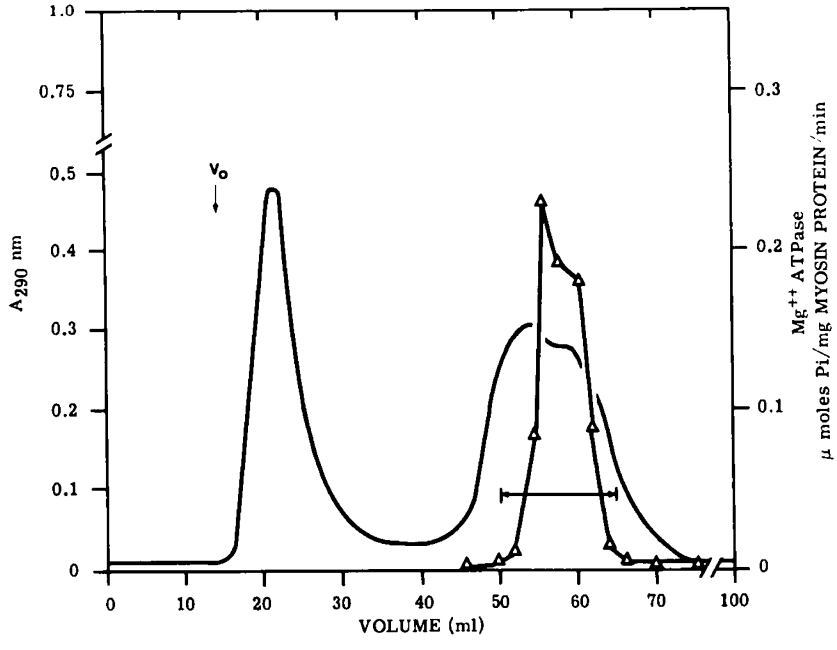

Fig. 1. Chromatography on Bio-Gel A-15 m, 200 to 400 mesh in 0.5 M KCl of the 20 to 50% saturated ammonium sulfate fraction of PMN leukocytes. The column (1.5 x 30 cm) was equilibrated with buffer (20 mM Tris-HCl, pH 7.2-0.5 M KCl-1 mM EDTA-2.5 mM DDT). The elution profile of absorbance at 290 nm (——) and of cofactor activity (——) are indicated. The Mg^{++}-ATPase activity of 0.015 mg rabbit PMN myosin and 0.18 mg/ml were assayed in the presence of added column fractions to determine the cofactor activity as μmoles P_i per mg myosin per min. V_o, void volume.

10% trichloroacetic acid-2% $Na_4P_2O_7$. The radioactivity associated with the precipitated protein was measured according to the method of Weller *et al.* (25). The sites of phosphorylation were determined by SDS gel electrophoresis. In experiments with electrophoretic analysis of the phosphorylated mixture, the reaction was terminated by addition of 1 ml 2% SDS and 1% $Na_4P_2O_7$. Then the samples were dialyzed against a solution of 0.2 M NaCl-0.1 M NaCl and deionized water for a total of 30 hr, renewing the solution as needed. Samples were then heated for 5 min at 100°C in the presence of 10% mercaptoethanol and 1% SDS and subjected to SDS polyacrylamide gel electrophoresis. At the completion of the electrophoresis, the gels were cut or subsequently stained with Coomassie brilliant blue R-250, destained, and scanned at 550 nm or cut into 3 mm slices with a gel cutter and transferred to a scintillation vial; 0.5 ml of 30% H_2O_2 was added and the vials containing the gels were heated at 50°C for 12 hr in a shaking water bath to dissolve the gel. Scintillation (Aquasol-2; New England Nuclear, Boston, MA) solution was added to the vials, and the samples were counted.

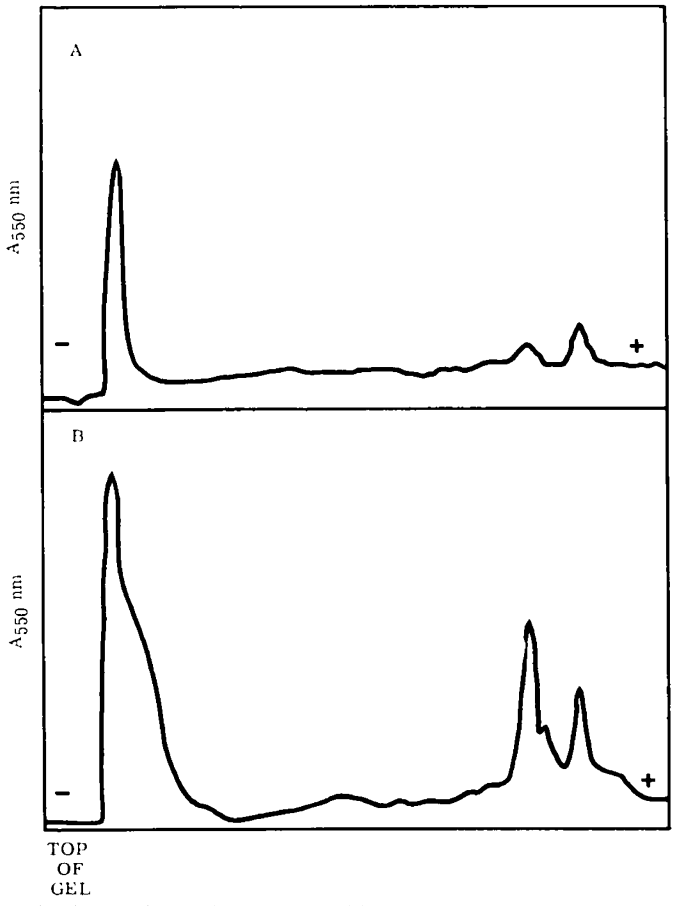

Fig. 2. Densitometric scans of purified myosin polypeptides were separated by electrophoresis on 7.5% polyacrylamide gels containing 1% SDS. Approximately 15 μg of proteins were stained with Coomassie blue and scanned at 550 nm. The heavy chain is at the left, followed by two light chains. *A*, purified PMN myosin; *B*, purified ALM myosin.

RESULTS

ISOLATION OF PMN AND ALM MYOSIN AND THEIR COFACTORS

Extracts of PMN and ALM were purified by ammonium sulfate fractionation and chromatography of the 20 to 50% ammonium sulfate fraction was performed on Bio-Gel A-15 m twice. The yield of purified PMN and ALM myosin was 4.6 ± 0.8 and 7 ± 1.5 mg (mean ± S.D.) per g of total cell protein for seven and three isolations, respectively. Like other myosins, purified PMN and ALM myosin had ATPase activity which was inhibited by Mg^{++}: 30 ± 6 and 15 ± 5 nmoles P_i/min/mg protein but activated by K^+-EDTA: 160 ± 26 and 102 ± 12 nmoles P_i/min/mg protein, respectively. The purity and subunit composition of purified PMN and ALM myosin were assessed by polyacrylamide gel electrophoresis in the presence of SDS. Myosins derived from both types of leukocytes consisted of one major band which comigrated with the heavy chain of muscle myosin (molecular weight, 200,000 daltons) and two other bands which possessed molecular weight of 20,000 and 16,000 daltons, respectively (Fig. 2, *A* and *B*).

Rabbit skeletal muscle F-actin activated the Mg^{++}-ATPase activity of purified rabbit PMN and ALM myosins if an additional cofactor, partially purified from the 20 to 50% saturated ammonium sulfate fraction of leukocyte extract supernatants on Bio-Gel A-15 m columns was added to the actomyosin (Figs. 1 and 3). In the presence of the respective cofactors, rabbit skeletal muscle F-actin increased the Mg^{++}-ATPase activity of purified rabbit PMN and ALM myosin by 11- and 8.5-fold, respectively (Table 1). The

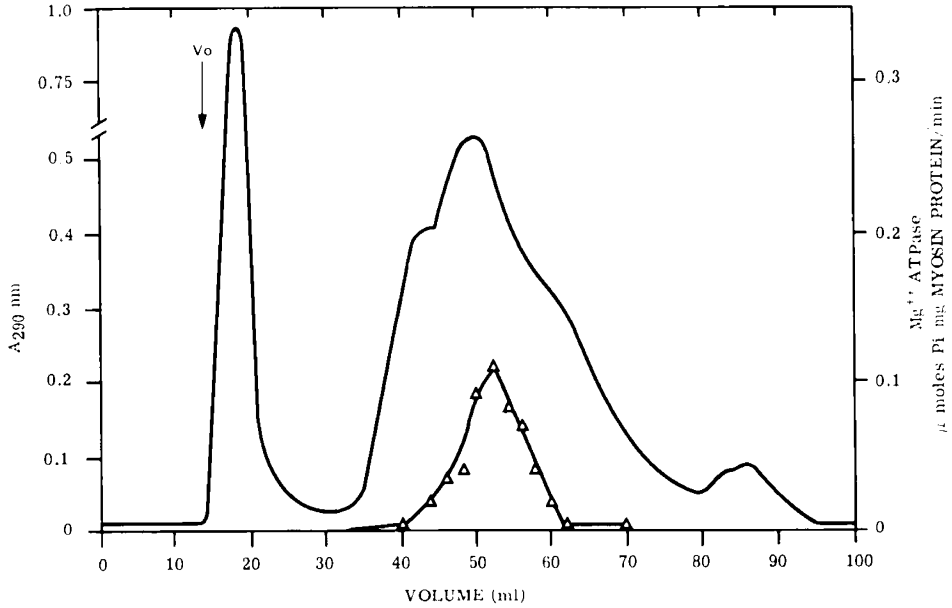

Fig. 3. Chromatography of the 20 to 50% saturated ammonium sulfate fraction of ALM extract supernatant in Bio-Gel A-15 m, 200 to 400 mesh in 0.6 M KCl. The elution profile of absorbance at 290 nm (——) and of cofactor activity (△) of 0.03 mg rabbit ALM myosin and 0.36 mg/ml rabbit skeletal muscle F-actin were assayed with the presence of added column fractions to determine the cofactor activity. V_0, void volume.

crude cofactors preparation isolated from the two types of leukocyte extracts were interchangeable with the leukocyte myosins. When ALM cofactor was added to a PMN actomyosin complex, it enhanced the Mg^{++}-ATPase activity of the PMN myosin by 3-fold greater than seen with the ALM cofactor and its own actomyosin complex. In contrast, addition of PMN cofactor did not enhance macrophage actomyosins Mg^{++}-ATPase activity beyond that observed with ALM cofactor and ALM actomyosin (Table 2).

Cofactor protein obtained from the PMN was further purified on DEAE-Sephacel at 4°C (Fig. 4). It was necessary to further purify crude cofactor within 5 days as its activity was lost after that time. It was found that the fraction after chromatography on DEAE-Sephacel was completed devoid of K^+-EDTA ATPase activity. Electrophoresis of this relatively pure fraction on SDS-polyacrylamide gels confirmed this observation by showing no evidence of the 200,000-dalton heavy chain of myosin. (Fig. 5).

Table 1. *Effect of rabbit skeletal F-actin and partially purified cofactor on the Mg^{++}-ATPase activity of rabbit PMN myosin and ALM myosin*[1]

Proteins	Mg^{++}-ATPase Activity (μmoles P_i/min/mg myosin protein)
PMN myosin	0.04
+ muscle F-actin	0.04
+ PMN cofactor	0.04
+ muscle F-actin + PMN cofactor	0.45
ALM myosin	0.02
+ muscle F-actin	0.02
+ ALM cofactor	0.02
+ muscle F-actin + ALM cofactor	0.17

[1] The concentrations of myosin and F-actin proteins in the ATPase assay were 0.02 and 0.1 mg/ml, respectively, and cofactor protein concentration was 0.1 mg/ml.

As shown in Figure 6, the activation of PMN myosin Mg^{++}-ATPase activity in the presence of rabbit skeletal F-actin by the 70,000-dalton protein was sigmoidal. The degree of activation of Mg^{++}-ATPase activity of PMN actomyosin was similar to that observed with the addition of crude cofactor. In the absence of Mg^{++}, the 70,000-dalton protein failed to enhance the activity of actomyosin. The Mg^{++}-dependent ATPase activity was not sensitive to removal of trace Ca^{++} by EGTA (data not shown).

PHOSPHORYLATION

To establish that the 70,000-dalton protein was a kinase, phosphorylation of PMN myosin was monitored (Fig. 7). In the presence of Mg^{++} and γ-labeled AT ^{32}P, phosphorylation of the 20,000-dalton light chain was observed. Further evidence that the myosin fraction was labeled with γ-^{32}P was obtained by observing the transfer of the γ-phosphate into trichloroacetic acid precipitable material. After a 30-min incubation, 0.62 ± 0.09 mole of

Table 2. *Interchangeable cofactor effect on peak PMN actomyosin or ALM actomyosin activity*[1]

Proteins	Mg^{++}-ATPase (μmoles P_i/min/mg myosin protein)
PMN myosin + F-actin + PMN cofactor	0.24 ± 0.01
PMN myosin + F-actin + AML cofactor	0.33 ± 0.04
ALM myosin + F-actin + ALM cofactor	0.13 ± 0.04
ALM myosin + F-actin + PMN cofactor	0.12 ± 0.01

[1] Bio-Gel A-15 m column fraction (0.1 ml) derived from the PMN or ALM was added to the respective actomyosin mixtures which contained 0.018 g/ml myosin and 0.18 mg/ml skeletal F-actin. The crude fraction containing peak cofactor activity is expressed by the increase in specific activity of either PMN or ALM myosin ATPase.

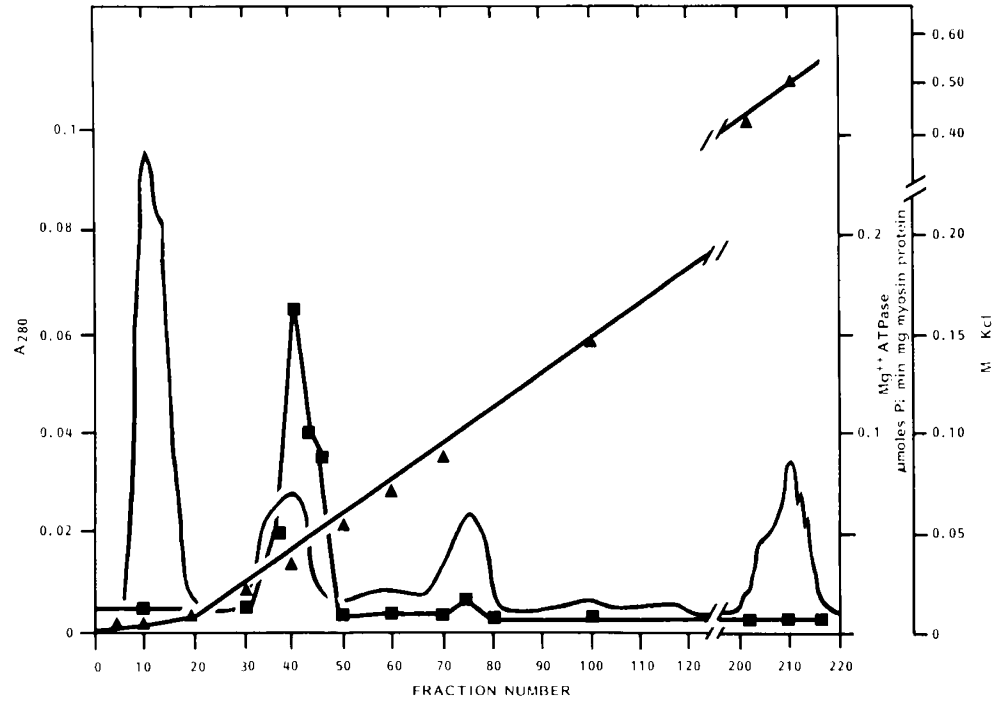

Fig. 4. DEAE-Sephacel chromatography of the pooled fraction from Bio-Gel A-15 m and dialyzed against starting buffer. Thirty mg of protein were applied in 4 ml to a 2.5 x 10 cm column of DEAE-Sephacel equilibrated with starting buffer (20 mM Tris-HCl, pH 8.0-1 mM DDT-0.34 M sucrose). The column was eluted with KCl gradient at a flow rate of 50 ml/hr. Fractions were collected in 3 ml/tube and absorbance at 280 nm (——) was monitored, (▲), molarity of KCl was determined; (■), cofactor activity was assayed by measuring actin-activated Mg^{++}-ATPase of PMN myosin.

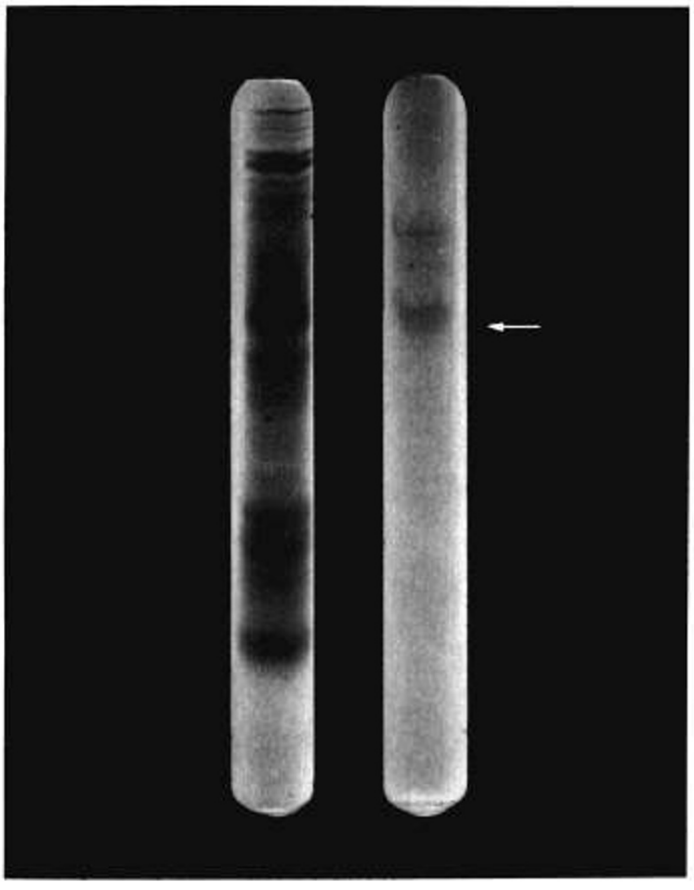

Fig. 5. The stained gels shown are electrophoretograms in sodium dodecyl sulfate of crude supernatant of homogenate protein (*left*) and of the purified protein kinase purified to 90% homogeneity by densitometer tracing (*right*) as indicated by *arrow*.

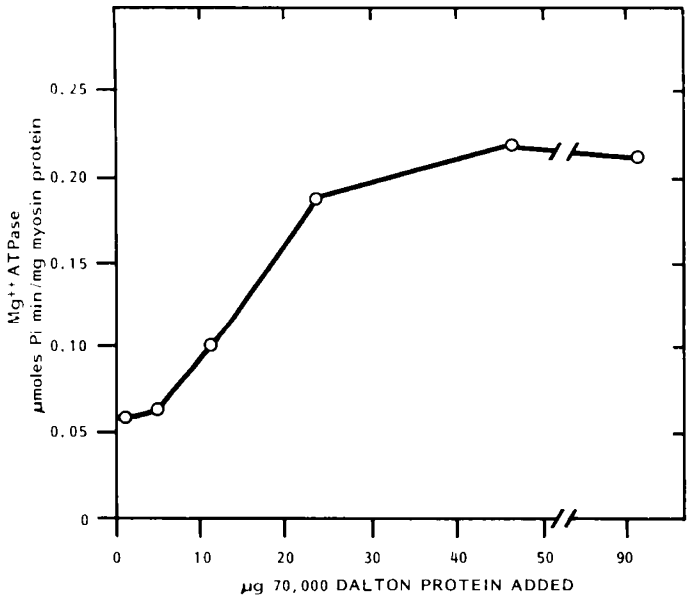

Fig. 6. Influence of kinase concentration on the Mg^{++}-dependent ATPase activity. One ml of the reaction mixture contained 15 μg PMN myosin; 150 μg F-actin and purified kinase as indicated. Assays were carried out as described in experimental procedure.

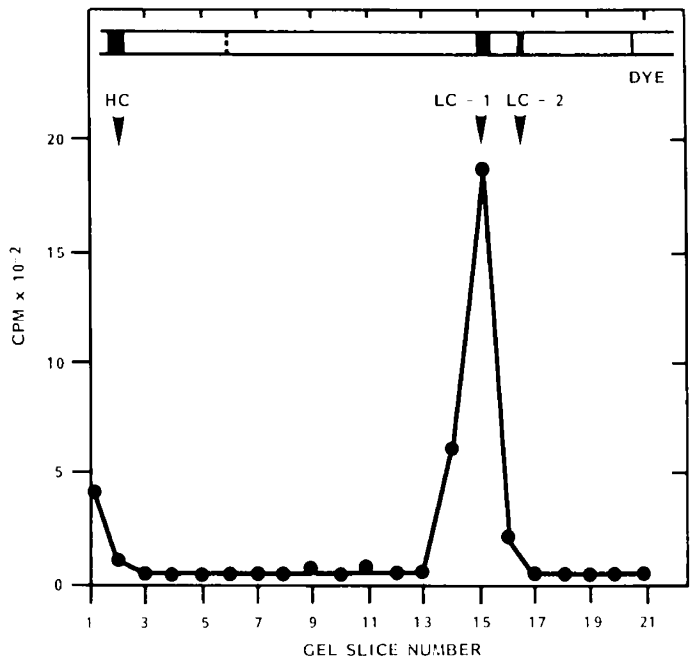

Fig. 7. Incorporation of ^{32}P into column purified PMN myosin after phosphorylation in the presence of column purified PMN light chain kinase. *HC*, myosin heavy chain (200,000 daltons); *LC*, myosin light chain, (20,000 and 16,000 daltons) are indicated.

phosphate was incorporated to each mole of myosin. Without the 70,000 dalton protein present, no incorporation of γ-^{32}P into trichloroacetic acid-precipitable material and sliced SDS-polyacrylamide gel was observed.

DISCUSSION

Several previous reports have established that the 20,000-dalton light chain of myosin from nonmuscle sources (1, 5, 8, 26) and smooth muscle cells (11) can be phosphorylated. Phosphorylation of the 20,000-dalton light chain is necessary for actin-induced activation of myosin Mg^{++}-ATPase activity (11). Studies with platelet, hamster, kidney, and brain light chain kinases have demonstrated that the enzyme is sensitive to Ca^{++} and the calcium-binding protein calmodulin (5, 8, 26). Other studies are in conflict with respect to the properties of the platelet kinase by concluding that the enzyme is not dependent on Ca^{++} for its activity (6).

The present study indicates that a Ca^{++}-insensitive myosin light chain kinase exists in rabbit PMN. It has a molecular weight of approximately 70,000, determined by polyacrylamide-SDS gel electrophoresis. This molecular weight is 10,000 less than that reported for a Ca^{++}-insensitive platelet myosin kinase (6).

The PMN myosin kinase is derived from the supernatant containing crude cofactor protein activity. Stossel and Hartwig (21) originally described a crude cofactor protein capable of activating myosin Mg^{++}-ATPase activity in rabbit alveolar macrophages which was not influenced by Ca^{++} ions. We confirmed their observations and found that the crude cofactor proteins isolated from both PMN and ALM were interchangeable in their activities with the respective cell myosins. Although the leukocyte crude cofactor proteins could potentiate the myosin Mg^{++}-ATPase activities of the leukocyte myosins, the PMN myosin consistently demonstrated more enzymatic activity than did ALM myosin. Whether this is an actual property of PMN myosin that could account for the enhanced motile and phagocytic responses of PMN compared to ALM needs to be further explored. Vertebrate fast and slow skeletal muscles are composed of fibers whose myosin is enzymatically and structurally distinct from each other (16). Enzymatically fast myosin is distinguishable from slow myo-

sin by severalfold higher specific actin-activated Mg^{++}-ATPase activity, which appears to correlate with light chain stoichiometry (16). No apparent difference, however, in the light chain molecular weight between PMN and ALM were noted which could account for the PMN myosin ATPase activity. It is possible that limited proteolysis of both PMN kinase and leukocyte myosins could account for the observed differences in myosin activities and Ca^{++} independency of the kinase in spite of the use in this study of protease inhibitors. In support of such a mechanism, the calmodulin-dependent cyclic adenosine 3',5'-monophosphate phosphodiesterase from bovine brain may be converted to an active, calmodulinand calcium-independent form by proteolytic degradation (12). It is also possible that the leukocyte myosin light chain kinase is truly independent of Ca^{++} as has been observed with a kinase derived from cultured aortic smooth muscle cells (4).

The identification of actin and myosin and related proteins in eukaryotic cells raises questions as to the mechanisms involved in the control of these proteins. The results obtained in this study indicate that phosphorylation of the 20,000 daltons light chain may have a crucial role in regulating actin-myosin interaction in phagocytic cells.

REFERENCES AND NOTES

1. Adelstein, R. S., and Conti, M. A.: Phosphorylation of platelet myosin increases actin-activated myosin ATPase activity. Nature (Lond.), *256:* 597 (1975).
2. Adelstein, R. S., Conti, M. A., and Hathaway D. R.: Phosphorylation of smooth muscle myosin light chain kinase by the catalytic subunit of adenosine 3',5' monophosphate-dependent protein kinase. J. Biol. Chem., *253:* 8347 (1978).
3. Boxer, L. A., and Stossel T. P.: Interactions of actin, myosin, and an actin-binding protein of chronic myelogenous leukemia leukocytes. J. Clin. Invest., *57:* (1976).
4. Chacko, S., Blose, S., and Adelstein, R. S.: In: F. A. Pepe: Motility in Cell Function. (Academic Press, Inc., New York, in press).
5. Dabrowska, R., and Hartshorne D. J.: A Ca^{2+}- and modulator-dependent myosin light chain kinase from non-muscle cells. Biochem. Biophys. Res. Commun., *85:* 1352 (1978).
6. Daniel J. L., and Adelstein R. S.: Isolation and properties of platelet myosin light chain kinase. Biochemistry, *15:* 2370 (1978).
7. Fairbanks, G., Steck, T. L., and Wallach, D. F. H.: Electrophoretic analysis of the major polypeptides of the human erythrocyte membranes. Biochemistry, *10:* 2006 (1971).
8. Hathaway, D. R., Adelstein, R. S.: Human platelet myosin light chain kinase requires the calcium-binding protein calmodulin for activity. Proc. Natl. Acad. Sci. U. S. A., *76:* 1653 (1979).
9. Hesketh, J. E., Virmaux, N., and Mandel P.: Evidence for a cyclic nucleotide-dependent phosphorylation of retinal myosin. FEBS Lett., *94:* 357 (1978).
10. Hitchcock, S. E.: Regulation of motility in nonmuscle cells. J. Cell Biol., *74:* 1 (1977).
11. Korn, E. D.: Biochemistry of actomyosin-dependent cell motility (a review). Proc. Natl. Acad. Sci. U. S. A., *75:* (1978).
12. Lin, Y. M., Lui, Y. P., and Cheung, W. Y.: Cyclic 3', 5'-nucleotide phosphodiesterase Ca^{++}-dependent formation of bovine brain enzyme-activation complex. FEBS Lett., *49:* 356 (1975).
13. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagents. J. Biol. Chem., *193:* 265 (1951).
14. Maruta, H., and Korn, E. D.: Acanthamoeba cofactor protein is a heavy chain kinase required for actin activation of the Mg^{2+}-ATPase activity of *Acanthamoeba* myosin I. J. Biol. Chem., *252:* 8329 (1977).
15. Myrvik, Q. N., Leake, E. S., and Fariss, B.: Studies on pulmonary alveolar macrophages from the normal rabbit: a technique to procure them in a high state of purity. J. Immunol., *86:* 128 (1967).
16. Pelloni-Mueller G., Ermini, M., and Jenny, E.: Myosin light chains of developing fast and slow rabbit skeletal muscle. FEBS Lett., *67:* 68 (1976).
17. Pollard, T. D., and Korn, E. D.: *Acanthamoeba* myosin. II. Interaction with actin and with a new cofactor protein required for actin activation of Mg^{++} adenosine triphosphatase activity. J. Biol. Chem., *248:* 4691 (1973).
18. Scordilis, S. P., and Adelstein, R. S.: Myoblast myosin phosphorylation is a prerequisite for actin-activation. Nature (Lond.), *268:* 558 (1977).
19. Spudick, J. A., and Watts, S.: The regulation of rabbit skeletal muscle contraction. I. Biochemical studies of the interaction of the tropomyosin-troponin complex with actin and the proteolytic fragments of myosin. J. Biol. Chem., *246:* 4866 (1971).
20. Stahelin, H., Karnovsky, M. L., Farnham, A. E., and Suter, E.: Studies on the interaction between phagocytes and tubercle bacilli. III. Some metabolic effects in guinea pigs associated with infection with tubercle bacilli. J. Exp. Med., *105:* 265 (1957).
21. Stossel, T. P., and Hartwig, J. H.: Interactions between actin, myosin, and an actin-binding protein from rabbit alveolar macrophages. J. Biol. Chem., *250:* 5706 (1975).
22. Stossel, T. P., and Pollard, T. D.: Myosin in polymorphonuclear leukocytes. J. Biol. Chem., *248:* 8288 (1973).
23. Trotter, J. A., and Adelstein, R. S.: Macrophage myosin. Regulation of actin-activated ATPase activity by phosphorylation of the 20,000-dalton light chain. J. Biol. Chem., *254:* 8781 (1979).
24. Weber, K., and Osborn, M.: The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. J. Biol. Chem., *244:* 4406 (1969).
25. Weller, M., Virmaux, N., and Mandel, P.: Light-stimulated phosphorylation of rhodopsin in the retina: the presence of a protein kinase that is specific for photobleached rhodopsin. Proc. Natl. Acad. Sci. U. S. A., *72:* 381 (1975).
26. Yerna, M. J., Dabrowska, R., Hartshorne, D. J., and Goldman, R. D.: Calcium-sensitive regulation of actin-myosin interactions in baby hamster kidney (BHK-21) cells. Proc. Natl. Acad. Sci. U. S. A., *76:* 184 (1979).

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