Myosin from pancreatic acinar carcinoma cells

Isolation, characterization and demonstration of heavy- and light-chain phosphorylation

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Myosin has been identified in a variety of non-muscle cells, and is believed to play a role in maintenance of cell shape, locomotion, cytokinesis, exocytosis and other cellular functions. In this paper we describe the purification of myosin from a pancreatic acinar-cell carcinoma of the rat which forms solid tumours, but retains many differentiated functions. The purified myosin was composed of a 200 000 Da heavy chain and two or three classes of light chains. Electron-microscopic examination of rotary-shadowed preparations revealed that individual molecules had two globular heads and a long tail measuring approx. 149 nm. The myosin was soluble in high-salt buffers and became sedimentable as the ionic strength was lowered. Examination of negative-stained preparations showed that this sedimentable myosin consisted of short, bipolar, thick filaments which had a strong tendency to aggregate in a head-to-head manner. The ATPase activity of the purified myosin was stimulated by EDTA or Ca^{2+} , but not by Mg^{2+} . In low ionic strength the Mg^{2+} -dependent ATPase activity was activated by muscle f-actin. The pancreatic myosin bound to actin and could be dissociated by the addition of MgATP . Myosin purified from cells cultured in media containing [^{32}P]P_i was phosphorylated on one of the light chains as well as the heavy chain. Thus pancreatic acinar cells contain a typical non-muscle myosin, and the subunits of this molecule are subject to post-translational modification by phosphorylation.

INTRODUCTION

Myosin has been found in a wide variety of eukaryotic non-muscle cells, including amoebae (Clarke & Spudich, 1974; Collins & Korn, 1981), platelets (Adelstein & Conti, 1975), macrophages (Hartwig & Stossel, 1975), brain (Burridge & Bray, 1975) and leukaemic myeloblasts (Sagara *et al.*, 1983). The cytoplasmic myosin may participate in certain cellular events, such as the maintenance of cell shape (Clarke & Spudich, 1977), cell motility (Korn, 1978), and the formation of the mitotic cleavage furrow (Fujiwara & Pollard, 1976). There is also evidence to suggest that myosin may play a role in endocytosis and exocytosis. Myosin associates with the cap structure which forms after ligand binding to cell-surface receptors (Condeelis, 1979; Bourguignon, 1980), and myosin localizes to the pseudopods of rabbit lung macrophages that are ingesting bacteria (Stendahl *et al.*, 1980). It has been suggested that myosin may play a role in the Ca^{2+} -dependent fusion of synaptic vesicles at the nerve terminus (Berl *et al.*, 1973), and Friedman *et al.* (1986) showed that injection of heavy meromyosin into the cytoplasm of adrenal chromaffin cells stimulated the secretion of catecholamines.

The precise role of myosin in stimulus–secretion coupling and its relationship to the maturation of the secretory apparatus during cytodifferentiation in exocrine cells is not understood. As an initial step toward elucidating the role of myosin in these processes, we have undertaken the purification and characterization of myosin from a transplantable pancreatic-acinar-cell carcinoma previously described by Reddy & Rao (1977). Normal pancreatic acinar cells contain a well-developed,

polarized, secretory apparatus, which has been extensively characterized (Palade, 1975; Jamieson, 1981), and differentiated cells of this transplantable acinar carcinoma exhibit a remarkable degree of polarity (Reddy & Rao, 1977). The preservation of an intact and functional secretory apparatus in this neoplasm (Warren, 1982; Iwanij & Jamieson, 1982; Becich & Reddy, 1982) provides a model system for investigating the role which myosin might play in secretion.

We have succeeded in purifying myosin from the acinar-cell tumour, and the structural and functional characteristics of this myosin were found to be similar to those of other non-muscle myosins. Of particular interest was the finding that both the heavy chain and the light chain of the acinar-cell myosin were phosphorylated *in vivo*. Since phosphorylation can regulate myosin activity (Adelstein & Conti, 1975; Trotter & Adelstein, 1979; Kuczmarski & Spudich, 1980; Scholey *et al.*, 1981; Ogihara *et al.*, 1983), it is possible that some acinar-cell functions may be controlled by myosin phosphorylation.

EXPERIMENTAL

Materials

Collagenase was obtained from Cooper Biomedical (Malvern, PA, U.S.A.). Soya-bean trypsin inhibitor, tosylphenylalanylchloromethane, tosyl-lysylchloromethane and benzamidine were from Sigma Chemical Co. (St. Louis, MO, U.S.A.). DTT was from US Biochemicals (Cleveland, OH, U.S.A.), leupeptin from Boehringer Mannheim Biochemicals (Mannheim, Germany) and [^{32}P]P_i (in aqueous solution) from Amersham

(Arlington Heights, IL, U.S.A.). Bio-Gel A-15m resin and glass chromatography columns were obtained from Bio-Rad Laboratories (Richmond, CA, U.S.A.). Rabbit back muscle was used to prepare actin (Pardee & Spudich, 1982) and myosin (Margossian & Lowey, 1982).

Animals

Male F-344 weanling rats (Harlan Industries, Madison, WI, U.S.A.) housed in individual cages were transplanted subcutaneously with small pieces of a previously described pancreatic-acinar-cell carcinoma (Reddy & Rao, 1977). Tumours that had grown to approx. 3 cm in diameter were used for the isolation of neoplastic acinar cells.

Myosin purification

Tumours were excised from rats and dissociated by the procedure of Amsterdam *et al.* (1978) as modified by Becich & Reddy (1982). Briefly, the tumour was minced, incubated with Krebs-Ringer Hepes buffer (pH 7.4) containing type IV collagenase, followed by Ca^{2+} - and Mg^{2+} -free buffer containing EDTA, and filtered through 125 μm nylon filter (Nitex) (Tetko, Elmsford, NY, U.S.A.). The dissociated cells were washed with buffer to remove red blood cells and then combined with 2 vol. of lysis buffer [10 mM-Tris (pH 7.5), 2 mM-EDTA, 1 mM-DTT, 40 mM-sodium pyrophosphate, 60% (w/v) sucrose, 1 mM each of phenylmethanesulphonyl fluoride, tosyl-lysylchloromethane, tosylphenylalanylchloromethane and benzamidine, 0.1 mg of soya-bean trypsin inhibitor/ml and 10 μg of leupeptin/ml]. The cells were lysed with ten passages of a glass/Teflon homogenizer, and whole cells, nuclei and other large particles were removed by centrifugation at 31 000 *g* for 20 min (Beckman JA-20 rotor). The supernatant was further purified by centrifugation at 105 000 *g* for 100 min (Beckman 70.1 Ti rotor).

The 105 000 *g* supernatant was dialysed at 4 °C versus 1.0 litre of 10 mM-triethanolamine buffer (pH 6.8)/0.5 mM-DTT/50 mM-KCl/1.0 mM-EDTA/0.5 mM-phenylmethanesulphonyl fluoride/1.0 mM- NaN_3 . The dialysis buffer was changed at 14 h, and after a total time of 20 h the contents of the dialysis bag were collected and centrifuged for 40 min at 25 000 *g*. The pellet was homogenized in an equal volume of buffer containing 20 mM-triethanolamine (pH 7.5), 2.0 mM-EDTA, 2.0 mM-DTT, 1.2 M-KI, 10 mM-ATP, 10 mM- MgCl_2 and 2.0 mM- CaCl_2 , and clarified by centrifugation (35 min at 100 000 *g*). Sucrose (0.1 g/ml) was added to the supernatant, and this solution was applied to a Bio-Gel A-15m column (2.5 cm $\times$ 90 cm) equilibrated first with column buffer [10 mM-triethanolamine (pH 7.5), 1.0 mM-EDTA, 0.6 M-KCl, 1.0 mM-DTT, 1.0 mM- NaN_3 and 0.2 mM-ATP], and then with 100 ml of a solution containing 10 mM-triethanolamine (pH 7.5), 1.0 mM-EDTA, 1.0 mM-DTT, 0.6 M-KI, 5.0 mM-ATP, 5.0 mM- MgCl_2 and 1.0 mM- CaCl_2 . The sample was applied and the column was then eluted with the previously described column buffer; 6 ml fractions were collected and assayed for protein content by the method of Bradford (1976). Myosin, which was eluted near the void volume, was detected either by ATPase activity or by the presence of the 200 000 Da heavy chain on SDS/PAGE. Appropriate fractions were pooled and concentrated with a collodion tube concentrator (Schleicher and Schuell) under

vacuum against 0.4 M-KCl/10 mM-triethanolamine (pH 7.5)/1.0 mM-DTT at 4 °C.

^{32}P -labelling

Freshly isolated tumour cells were dissociated as above, and the cells were incubated in 50 ml of phosphate-free Krebs-Ringer Hepes buffer at 37 °C for 45 min. Carrier-free [^{32}P]P_i (5 mCi) was then added, and, after an additional incubation of 60 min, cells were lysed and myosin was purified as described above. Although not determined in the present study, it has been shown that under similar conditions intracellular ATP pools are equilibrated with the media within 45 min (Garrison *et al.*, 1981). The specific radioactivity of the [^{32}P]ATP was determined as outlined by Garrison (1978). Briefly, 250 μl of cell suspension was sedimented by brief centrifugation (12 000 *g*, 1 min) and resuspended in 750 μl of 0.6 M- HClO_4 and then centrifuged again. The supernatant was transferred to an ice-cold test tube, neutralized with 150 μl of 3.0 M- KHCO_3 , and stored at -50 °C. ATP was subsequently purified from the supernatant by h.p.l.c., on a Whatman Partisil 10 strong anion-exchange column (Whatman Chemical Separation, Clifton, NJ, U.S.A.).

To calculate specific radioactivity, the concentration of the purified ATP was determined by measuring the A_{259} on a Beckman DU-7 spectrophotometer, and radioactivity was measured with a Beckman LS 6800 scintillation counter. To verify purity of the ATP after h.p.l.c., a 2 μl sample was separated on a polyethyleneimine plate (J. T. Baker Chemical Co., Phillipsburg, NJ, U.S.A.) which was developed with 1.5 M-LiCl in an Eastman Chromagram Apparatus (Eastman Kodak Co., Rochester, NY, U.S.A.). An autoradiogram of the t.l.c. plate revealed that over 90% of the radioactivity was coincident with the ATP standard and that the remainder migrated with P_i.

To quantify heavy- and light-chain phosphorylation, protein bands separated by SDS/PAGE were cut from the gel, hydrolysed in 30% (v/v) H_2O_2 at 80 °C for 60 min, and the amount of radioactivity was measured with a scintillation counter. To determine the ratio of heavy- to light-chain phosphorylation, purified myosin was subjected to SDS/PAGE and autoradiograms were scanned with an LKB Ultrascan XL laser densitometer.

Assay for ATPase activity

ATPase activity of the purified myosin was measured by the colorimetric assay of Lanzetta *et al.* (1979). Myosin concentration was 44 $\mu\text{g}/\text{ml}$ and the ATP concentration was 1.0 mM. The following final buffer concentrations were used: calcium buffer [0.6 M-KCl, 10 mM-Tris (pH 8.0) and 10 mM- CaCl_2]; EDTA buffer [0.5 M-KCl, 2.0 mM-EDTA and 15 mM-triethanolamine (pH 7.5)]; magnesium buffer [15 mM-triethanolamine (pH 7.5) and 4.0 mM- MgCl_2]. Actin-activated ATPase activity was determined by adding rabbit muscle actin (9.0–270.0 $\mu\text{g}/\text{ml}$) to a reaction mixture containing the myosin in 15 mM-KCl/0.1 mM- CaCl_2 /5 mM- MgCl_2 /1.0 mM-ATP/25 mM-Mes (pH 7.0). All reactions were carried out at 25 °C and were linear with time.

Assembly assays

Myosin was extensively dialysed against 10 mM-triethanolamine (pH 7.5)/0.5 mM-DTT/25 mM-KCl and

assayed at a final concentration of 0.2 mg/ml. Myosin assembly was measured by a sedimentation assay using a Beckman Airfuge. To eliminate non-specific protein binding, the centrifuge tubes were preincubated with bovine serum albumin (1.0 mg/ml) for 60 min, washed three times with 0.5 M-KCl, three times with distilled water and then air-dried overnight. The myosin solution (final volume 100 μ l) was incubated for 10 min at 25 °C in the treated polypropylene centrifuge tubes (Robbins Scientific, Palo Alto, CA, U.S.A.) and then centrifuged in a Beckman Airfuge [10 min at 160 kPa (23 lb/in²), room temperature]. Duplicate 40 μ l portions were removed from the upper portion of the supernatant fraction, and the protein concentration was determined. The fraction of soluble myosin was calculated by comparison with the measured protein concentration of an identical sample which had not been centrifuged. This value was subtracted from 1.0 to obtain the fraction of assembled myosin.

Electron microscopy

Rotary shadowing. Purified myosin, at a concentration of 0.05 mg/ml, was dialysed against 50% glycerol/5.0 mM-triethanolamine (pH 7.5)/50 mM-KCl. The myosin was sprayed on to freshly cleaved mica as described by Tyler & Branton (1980) and air-dried at room temperature. The samples were rotary shadowed with platinum, carbon-coated and processed for examination in the electron microscope.

Negative staining. A drop of the protein solution was applied to a 300-mesh copper grid coated with Formvar and carbon. After 30 s the grid was washed with five drops of aq. 1.5% (w/v) uranyl acetate. The stain was drained with a strip of filter paper and the grid was examined in a JEOL 100 S electron microscope operated at 60 kV. The buffer for myosin-filament formation was 10 mM-Mes (pH 6.8)/50 mM-KCl/5.0 mM-MgCl₂ and the myosin concentration was 0.18 mg/ml. For demonstration of arrowheads, rabbit muscle f-actin (0.02 mg/ml) in Buffer K (0.3 M-KCl, 2.0 mM-MgCl₂, 10 mM-triethanolamine, pH 7.5) was applied to the grid and after 30 s was rinsed with five drops of Buffer K. A drop of pancreatic myosin (0.11 mg/ml in Buffer K) was then applied to the grid and allowed to bind to the f-actin for 60 s. The grids were then washed with five drops of either Buffer K or Buffer K containing 1.0 mM-ATP and then negatively stained.

Two-dimensional t.l.c.

We used the method of Cooper *et al.* (1983) to determine which amino acids were phosphorylated. Briefly, 10 μ l of hydrolysed ³²P-labelled pancreatic myosin (representing ~ 800 c.p.m.) was spotted on the t.l.c. plate along with unlabelled standards. After electrophoresis the standards were developed with ninhydrin and the ³²P-labelled amino acids were identified by autoradiography.

RESULTS

Purification

Cells were lysed by gentle homogenization in a sucrose/pyrophosphate buffer, which has been shown to limit proteolysis of cytoskeletal proteins (Uyemura *et al.*,

Fig. 1. SDS/PAGE and ³²P-autoradiogram of myosin obtained from radiolabelled pancreatic tumour cells

Dissociated tumour cells were labelled with ³²P as described in the Experimental section, and myosin was purified from these cells. Coomassie Brilliant Blue-stained SDS/PAGE of the purified myosin (CBB) and the corresponding autoradiogram (³²P) are shown. The heavy chain and one of the light chains were phosphorylated. Molecular masses (in kDa) are shown to the left of the Figure.

1978). In addition, a mixture of proteinase inhibitors was included in the lysis buffer. The pancreatic myosin was soluble in this buffer, but after dialysis against low ionic strength it formed a complex which could be sedimented at relatively low speed. SDS/PAGE revealed that the sedimentable material contained a variety of proteins in addition to myosin. Although actin was a component of this complex, a large amount of actin remained soluble. This did not appear to influence the efficiency of myosin precipitation, however, since the addition of excess rabbit muscle f-actin did not increase the amount of myosin that precipitated (results not shown). Myosin was separated from associated proteins by gel-filtration chromatography in the presence of KI. Up to 0.5 mg of highly purified myosin could be obtained from two tumour-bearing rats (~ 5 ml of washed packed cells).

When assayed by SDS/PAGE, the purified myosin always contained a heavy chain which co-migrated with rabbit skeletal-muscle myosin and thus had a molecular mass of ~ 200 000 Da. In addition, a variable number of light chains were associated with the heavy chain. In our cleanest preparations, two major light chains with molecular masses of 21 500 and 17 000 Da were observed. Some preparations, however, contained bands of lower molecular mass, which may represent breakdown products (Fig. 1). Electron-microscopic examination after platinum shadowing revealed that the purified myosin molecules had a typical asymmetric shape. As shown in Fig. 2(a), the pancreatic myosin was composed of two globular 'heads' attached to a long tail measuring 149 ± 16 nm.

ATPase activity

Like myosins isolated from other sources, the pancreatic myosin had a distinctive ATPase activity profile. In high salt (0.5 M-KCl) the purified myosin displayed high ATPase activity when EDTA or Ca²⁺ was present.

Fig. 2. Electron-microscopic examination of the purified myosin

Panel (a) demonstrates representative molecules of the pancreatic myosin examined by rotary shadowing. Panel (b) shows bipolar thick filaments produced by self-assembly of the myosin molecules, and panels (c) and (d) demonstrate myosin interaction with filamentous actin in the absence (c) and the presence (d) of ATP. Magnification: (a) $\times 66\,000$; (b) $\times 19\,900$ (inset, $\times 61\,900$); (c) $\times 61\,900$; (d) $\times 54\,500$.

In the presence of Mg^{2+} , however, the ATPase activity was much lower (Table 1). In low salt (20 mM-KCl) the myosin MgATPase activity was activated by the addition of purified rabbit muscle f-actin. As shown in Table 1, an actin activation of about 9-fold was measured.

Myosin self-assembly

In high salt (> 0.4 M-KCl) the pancreatic myosin was soluble, as demonstrated by the fact that protein was not sedimented by centrifugation at $100\,000\ g$ for 10 min. As the salt concentration was lowered, however, the myosin became sedimentable, and half-maximal assembly occurred at a salt concentration of ~ 175 mM (Fig. 3). Similar results were obtained with rabbit skeletal-muscle myosin (Fig. 3). Examination in the electron microscope indicated that in low ionic strength the pancreatic myosin had assembled into bipolar thick filaments with an average length of $0.37\ \mu\text{m}$ (Fig. 2b). These individual thick filaments tended to aggregate side-to-side as well as head-to-head.

Actin-myosin interaction

Actin activation of Mg^{2+} -dependent myosin ATPase activity implied that these two proteins could interact. It was possible to directly demonstrate this binding by electron microscopy. As shown in Fig. 2(c), the pancreatic myosin decorated f-actin filaments and formed the typical

Table 1. ATPase activity of the pancreatic-tumour myosin

Myosin was assayed to determine ATPase activity in the presence of Ca^{2+} , Mg^{2+} and EDTA. The activity is expressed as $\mu\text{mol}/\text{min}$ per mg of myosin and was determined as outlined in the Experimental section. Also included is the ATPase activity of the myosin in response to increasing concentration of actin.

Additions (see the Experimental section)	Specific activity ($\mu\text{mol}/\text{min}$ per mg of protein)	
Mg^{2+}	0.0682	
Ca^{2+}	0.2045	
EDTA	0.2273	

Actin added ($\mu\text{g}/\text{ml}$)	ATPase activity	
	($\mu\text{mol}/\text{min}$ per mg)	(fold increase)
0	0.0071	1.00
9.0	0.0081	1.15
18.0	0.0209	2.94
90.0	0.0387	5.45
180.0	0.0501	7.06
270.0	0.0636	8.96

arrowhead pattern. As with muscle myosins, the pancreatic myosin was dissociated from the actin by the addition of MgATP (Fig. 2d).

Myosin phosphorylation *in vivo*

In skeletal- and smooth-muscle as well as in many different non-muscle myosins, the light chain is phosphorylated, and often this post-translational modification can influence myosin assembly and ATPase activity (Adelstein & Conti, 1975; Trotter & Adelstein, 1979; Scholey *et al.*, 1980). More recently, it has been demonstrated that the heavy chain of some non-muscle myosins can also be phosphorylated (Kuczmarski & Spudich, 1980; Collins & Korn, 1980; Fechner & Cebra, 1982; Sagara *et al.*, 1983). To determine if the pancreatic myosin light and/or heavy chains were phosphorylated *in vivo*, cells were incubated in media containing $[^{32}\text{P}]\text{P}_i$. Myosin was purified, fractionated by SDS/PAGE, and analysed by autoradiography. As shown in Fig. 1, one of the light chains as well as the heavy chain were phosphorylated *in vivo*. This pattern was observed in three independent myosin preparations. Densitometric scans of the autoradiograms revealed that the ratio of phosphorylation (heavy chain:light chain) ranged from 4.7:1 to 12.2:1. In one preparation the specific radioactivity of the intracellular ATP pool was calculated as described in the Experimental section. From this, it was possible to calculate that during the 60 min incubation with $[^{32}\text{P}]\text{P}_i$ the myosin had been phosphorylated to the extent of 0.053 mol of P_i/mol of heavy chain and 0.007 mol of P_i/mol of light chain. To determine which amino acid was phosphorylated, intact myosin was hydrolysed with 0.6 M-HCl and the hydrolysate was analysed by two-dimensional thin-layer

Fig. 3. Pancreatic-tumour myosin self-assembly in the presence of increasing KCl concentration

Duplicate samples of the purified myosin from pancreatic carcinoma cells (◆) and rabbit muscle (□) were assayed for their ability to assemble into sedimentable filaments at various concentrations of KCl as described in the Experimental section.

electrophoresis. Most of the radioactivity was associated with serine, but a small amount of threonine was labelled (results not shown). There was no evidence of tyrosine phosphorylation.

DISCUSSION

To elucidate the role of myosin in the secretory cells of the rat exocrine pancreas, we have developed a protocol for the isolation of this contractile protein from an acinar-cell carcinoma. The use of a well-differentiated carcinoma (Reddy & Rao, 1977) rather than normal rat pancreas has allowed us to purify myosin from a single cell type. These tumour cells retain several differentiated functions, including the ability to synthesize and package a full complement of secretory proteins (Hansen *et al.*, 1983) and to respond to secretagogues (Iwanij & Jamieson, 1982; Warren, 1982).

The purified acinar-cell myosin resembled other non-muscle myosins in terms of subunit composition, molecular shape and ATPase activity. By SDS/PAGE, the purified myosin molecule consisted of a heavy chain of ~200 000 Da and two light chains of ~21 500 and 17 000 Da. The organization of these subunits in the native molecule was identical with that of other myosins; it consisted of a long filamentous tail of ~149 nm and two globular heads. Activation of ATPase activity in the presence of EDTA and Ca^{2+} , but not Mg^{2+} , has been reported for other vertebrate non-muscle myosins, including those of human platelets (Adelstein *et al.*, 1971), rabbit macrophages (Stossel & Hartwig, 1975) and rat hepatocytes (Brandon, 1976). Like other myosins, the Mg^{2+} -dependent ATPase activity measured in low ionic strength was activated several-fold by f-actin. Myosin isolated from normal rat pancreas (Vandermeers *et al.*, 1982) exhibited ATPase activity similar to that seen in our preparations. However, detailed characterization of normal rat pancreatic myosin (e.g. the

structural characterization, self-assembly, actin binding and phosphorylation) has, to the best of our knowledge, not been reported.

The ability of tumour myosin to interact with actin was demonstrated by electron microscopy. The pancreatic myosin bound to actin filaments to form the familiar 'herring-bone' pattern (Huxley, 1963), and MgATP addition caused myosin dissociation (Figs. 2c and 2d). Like other myosins (Pollard & Weihing, 1974), the pancreatic myosin was soluble in high salt, but became sedimentable as the ionic strength was lowered, with half-maximal assembly occurring at ~150 mM-KCl (Fig. 3). When examined in the electron microscope, it was apparent that the myosin had assembled into typical bipolar thick filaments, and these filaments had a strong tendency to interact in a head-to-head manner. Similar head-to-head aggregates have been reported for myosin purified from brain (Burridge & Bray, 1975; Kuczmarski & Rosenbaum, 1979) and *Acanthamoeba* (Collins *et al.*, 1982).

For the cell to carry out its various contractile functions, it presumably regulates the state of assembly, location and enzymic activity of the myosin molecules. In non-muscle cells, phosphorylation plays a major role in this regulation. In all systems so far studied, light-chain phosphorylation plays a stimulatory role and can activate the actin-activated ATPase activity as well as enhance filament assembly (Adelstein & Conti, 1975; Trotter & Adelstein, 1979; Scholey *et al.*, 1980, 1981; Sagara *et al.*, 1983; Citi & Kendrick-Jones, 1986). More recently it has become apparent that the heavy chain can also be phosphorylated in non-muscle cells. With this post-translational modification, however, the effect on myosin function varies from system to system. In *Dictyostelium* (Kuczmarski & Spudich, 1980) and *Acanthamoeba* (Collins & Korn, 1980) heavy-chain phosphorylation inhibits both assembly and actin-activated ATPase activity, whereas in *Physarum* heavy-chain phosphorylation enhances these functions (Ogihara *et al.*, 1983).

Although both heavy- and light-chain phosphorylation are known to occur in several non-muscle myosins, few studies have carefully examined the interaction between these subunits. Myosins isolated from chicken intestinal brush border (Rieker *et al.*, 1987) or cow brain (Tanaka *et al.*, 1986) are stimulated by light-chain phosphorylation. Similar results are obtained if light and heavy chains are phosphorylated simultaneously, suggesting that in these systems heavy-chain phosphorylation is not inhibitory. Unfortunately, it has not been possible to study the effect of heavy-chain phosphorylation alone in these systems. Heavy-chain phosphorylation can, however, inhibit the stimulatory effects resulting from light-chain phosphorylation in leukaemic-myeloblast myosin (Sagara *et al.*, 1983) and *Dictyostelium* myosin (E. R. Kuczmarski, unpublished work).

The pancreatic myosin described in the present study provides another example of a non-muscle myosin which has both heavy and light chains phosphorylated *in vivo*. As isolated, the myosin can assemble and does have reasonable ATPase activity. Thus it should be possible to measure changes in myosin function brought about by different extents of subunit phosphorylation. To carry out these studies, it will first be necessary to identify specific kinases and phosphatases. The measured extents of phosphorylation are rather low, and this may be due

to technical factors. For example, the cells were only labelled for 60 min, and if the rate of phosphate turnover *in vivo* is slow, a majority of the molecules could have retained unlabelled phosphate, leading to an underestimate of the true stoichiometry. In addition, it is possible that the myosin was partially dephosphorylated during the lengthy purification procedure.

Exocytosis is one of the cellular activities which a myosin-linked regulatory system might control. Actin-myosin interaction may produce the mechanical force necessary to allow the secretion of membrane-bound intracellular material. In support of this hypothesis, phosphorylation of the myosin light chain has been correlated with the secretion of platelet dense granules after the addition of thrombin (Daniel *et al.*, 1981). Activation of platelets with arachidonic acid also causes an increase in light-chain phosphorylation (Cox *et al.*, 1984). The demonstration that chromaffin cells are induced to secrete by the introduction of heavy meromyosin (Friedman *et al.*, 1986) also implies a role for myosin in exocytosis. Because these pancreatic acinar cells respond to secretagogues, it should be possible to determine if changes in the heavy- and/or light-chain phosphorylation are correlated with secretion. In addition, it will be of interest to monitor changes in myosin location and phosphorylation during extracellular-matrix-mediated modulation of differentiation (Watanabe *et al.*, 1984) and morphogenesis (Reddy *et al.*, 1986).

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