MYOSINS OF SECRETORY TISSUES

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ABSTRACT

Myosin has been purified from the principal pancreatic islet of catfish, hog salivary gland, and hog pituitary. Use of the protease inhibitor Trasylol (FBA Pharmaceuticals, New York) was essential in the isolation of pituitary myosin. Secretory tissue myosins were very similar to smooth muscle myosin, having a heavy chain of 200,000 daltons and light chains of 14,000 and 19,000 daltons. Salivary gland myosin cross-reacted with antibodies directed toward both smooth muscle myosin and fibroblast myosin, but not with antiskeletal muscle myosin serum. The specific myosin ATPase activity measured in 0.6 M KCl was present. Tissues associated with secretion of hormone granules contained substantial amounts of this ATPase, rat pancreatic islets having 4.5 times that of rat liver. Activation of low ionic strength myosin ATPase by actin could not be demonstrated despite adequate binding of the myosin to muscle actin and elution by MgATP. The myosins were located primarily in the cytoplasm as determined by cell fractionation and were quite soluble in buffers of low ionic strength.

KEY WORDS myosin · secretory · islet · pituitary

A large body of evidence implies that the secretory extrusion of hormone granules is an energy-requiring process (4) which utilizes a microfilamentous-microtubular system, a network of actin-like microfilaments and microtubules (15, 33, 20, 19, 14). The mechanism by which the microfilamentous-microtubular system results in secretion is not known, but an analogy to muscle is apparent: actin and myosin interact in the presence of ATP to generate movement. The presence of actin-like microfilaments in islet tissue (20, 12) and their apparent relationship to secretion (33) suggested that myosin might exist in secretory tissues. A putative role of myosin would be that of transducing the chemical energy of ATP into the mechanical energy of secretion. Understanding the mechanism of secretion of peptide hormone-containing granules is of potential significance in elucidating the pathogenesis of such diseases as adult-onset diabetes mellitus where a principal defect appears to be the inappropriately small release of insulin in response to glucose (23) despite available pancreatic insulin stores which are sometimes normal (34).

In this paper, we present the identification, purification, quantitation, and properties of soluble myosins in secretory tissues.

MATERIALS AND METHODS

Tissues and Reagents

Principal pancreatic islets of the channel catfish, *Ictalurus punctatus*, were dissected from commercially farmed fish (Lambrich Brothers' Co., Route 2, Imperial, Mo.) which had been freshly killed by a blow on the head. Hog tissues were obtained from a local slaughterhouse and transported to the laboratory on ice within 3 h of the death of the animal. Hog parotid was separated carefully from overlying platysma muscle. Sprague-Dawley rats were employed. The identity of tissues was

confirmed by histological section. Trasylol (aprotinin) was purchased from FBA Pharmaceuticals, New York. Enzymes and substrates were purchased from Sigma Chemical Co., St. Louis, Mo. Twice-polymerized actin was prepared from rat or rabbit back and hindlimb muscle without chromatographic purification (29).

Assays

Myosin ATPase was determined from phosphate generation over 30–60 min at 37°C in an assay containing 10 mM imidazole (pH 7.0), 2 mM EDTA, 0.6 M KCl, and 2 mM ATP with a final volume of 2.2 (3) or 0.55 ml. Conditions for actomyosin ATPase were 10 mM imidazole (pH 7.0), 2.7 mM $MgCl_2$, 1 mM ATP, and 0–60 mM KCl. To conserve enzyme, in some experiments ATPase was determined from phosphate liberated and ADP produced using the enzymatic fluorometric methods of Lowry and Passonneau (16). The reaction conditions described above were used in a final volume of 100 μl. The reaction was stopped by placing 10-μl aliquots on ice and then heating at 65°C for 10 min to destroy enzymatic activity. Phosphate was measured by NADPH produced from the following reactions:

$$\text{glycogen} + P_i \xrightarrow{\text{phosphorylase}} \text{glu-1-P}$$

$$\text{glu-1-P} \xrightarrow{\text{phosphoglucomutase}} \text{glu-6-P}$$

$$\text{glu-6-P} + \text{NADP} \xrightarrow{\text{glu-6-P dehydrogenase}} \text{6-phospho-gluconolactone} + \text{NADPH} + H^+.$$

ADP was measured by NADH consumed in the following reactions:

$$\text{ADP} + \text{phosphoenolpyruvate} \xrightarrow{\text{pyruvate kinase}} \text{ATP} + \text{pyruvate}$$

$$\text{pyruvate} + \text{NADH} \xrightarrow{\text{lactate dehydrogenase}} \text{lactate} + \text{NAD}.$$

This method is capable of measuring 0.1 nmol of ADP or P_i. ATPase activity is expressed as nanomoles of P_i liberated per minute.

Sodium dodecyl sulfate (SDS)[1] polyacrylamide gel electrophoresis was carried out in the neutral P_i system of Maizel (18). Coomassie Blue-stained (Sigma-Aldrich, Inc., St. Louis, Mo.) gels were quantitated using a gel scanner (Gilford Instrument Laboratories, Inc., Oberlin, Ohio). Protein was determined by the method of Lowry et al. (17).

Pituitary Cell Fractionation

Hog pituitaries were mixed with 2 vol of 0.3 M sucrose containing 50 mM Tris-Cl (pH 7.4) (ST buffer) and homogenized eight strokes with a motor-driven, loose-fitting, Teflon-glass homogenizer. All operations were performed at 4°C. The homogenate was centrifuged at 3,000 *g* for 10 min and the pellet was washed with 2 vol of ST buffer. The supernates were combined and centrifuged at 41,000 *g* for 30 min, and the 41,000-*g* supernate was then centrifuged at 105,000 *g* for 60 min. The 41,000-*g* pellet was taken up in aqueous sucrose to give a final density of 1.18, and secretory granules were prepared by the discontinuous sucrose gradient procedure described by Poirier et al. (25). Granule membranes were prepared by hypotonic lysis. Purified granules were suspended in a 10-fold excess of distilled water and, after 60 min of stirring at 4°C, the solution was centrifuged for 1.5 h at 25,000 rpm in an SW 27 rotor using a discontinuous sucrose gradient. Granule membranes were concentrated at the water–1.14-density sucrose and 1.14–1.18-density sucrose interfaces. These interfaces were removed, the samples were diluted 1:4 in water, and the membranes were collected by sedimentation at 45,000 *g*.

Immunologic Studies

Antibodies were prepared as described in references 35 and 21. Immunodiffusion was performed in Ouchterlony plates (Miles Laboratories, Elkhart, Ind.).

RESULTS

Myosin ATPase

Myosin has a unique ATPase which is activated by 0.6 M KCl in the presence of EDTA whereas other ATPases are inhibited under these conditions (28, 21). This property is a useful probe for cell myosins present in small concentrations (21). Myosin ATPase is defined as the activity measured in 0.6 M KCl, 2 mM EDTA, 2 mM ATP, and 10 mM imidazole (pH 7.0) (see Materials and Methods). The results of myosin ATPase activity measurements in a variety of tissues are listed in Table I in order of decreasing ATPase content. Rat muscle exhibits the expected large amount, but several secretory tissues, such as salivary gland and pancreatic islets, contain surprisingly large amounts of myosin ATPase despite the fact that much of the cell protein consists of stored secretory products. Rat islets contain 4.5 times ($P < .05$) the myosin ATPase of rat liver. Likewise, hog pituitary has 4.6 times ($P < .01$) the myosin

[1] *Abbreviations used in this paper:* DTT, dithiothreitol; $K_d = (V_e - V_0)/(V_s - V_0)$ where V_e = elution volume, V_0 = void volume, and V_s = volume of salt peak; KT buffer, 0.6 M KCl, 15 mM Tris-Cl (pH 7.0), containing 2 mM DTT; KT-AM buffer, KT buffer containing 0.5 mM NaATP and 0.5 mM $MgCl_2$; SDS, sodium dodecyl sulfate; ST buffer, 50 mM Tris-Cl, 0.3 M sucrose (pH 7.4).

TABLE I

Myosin ATPase Content of Various Tissues

Tissue	ATPase activity
	nmol/min/mg tissue protein
Rat skeletal muscle	638.6 ± 47.8
Hog submaxillary gland	5.13 ± 0.70
Catfish islet	3.90 ± 0.26
Rat lung	2.98 ± 0.36
Rat islet	2.80 ± 0.65
Rat submaxillary gland	2.63 ± 0.30
Hog parotid	2.62 ± 0.38
Hog adrenal cortex	2.44 ± 0.11
Hog adrenal medulla	2.33 ± 0.20
Rat spleen	2.15 ± 0.40
Rat adrenal	1.60 ± 0.06
Rat whole pancreas	1.13 ± 0.14
Rat kidney	1.06 ± 0.27
Hog anterior pituitary	0.96 ± 0.02
Hog posterior pituitary	0.80 ± 0.06
Rat liver	0.62 ± 0.17
Hog thyroid	0.48 ± 0.23
Rat brain	0.37 ± 0.07
Hog cerebrum	0.19 ± 0.04

Tissues were homogenized in 5–20 vol of KT buffer and incubated at 0°C for 1 h. An aliquot was removed for protein determination, and the homogenate was centrifuged at 10,000 *g* for 10 min. Myosin ATPase was determined in the supernate after dialysis against KT buffer. Three to five preparations of each tissue were studied. All values are mean ± SEM.

ATPase of hog cerebrum. Thyroid, liver, and brain were consistently low in ATPase; none of these tissues contains secretory granules, although microvesicles are present. These differences in myosin ATPase activity are not due to variations in tissue proteolytic enzyme activity inasmuch as values for brain and whole pancreas obtained after 24 h of dialysis against 0.6 M KCl, 15 mM tris-Cl (pH 7.0), with 2 mM dithiothreitol (KT buffer) agreed with values obtained after only 4 h of dialysis. In addition, extraction of brain with buffers containing 100 U/ml of the protease inhibitor Trasylol resulted in no increase in myosin ATPase activity.

Purification of Myosin

To confirm that the K^+-EDTA ATPase measured was in fact due to myosin, a procedure useful for fibroblast myosin was used (3). The ATPase was purified from hog parotid, the principal pancreatic islets of catfish, and hog pituitary. The preparative scheme is illustrated with parotid tissue. Hog parotid glands were homogenized in buffer containing 0.6 M KCl, and the resulting extract was fractionated with ammonium sulfate and chromatographed on Sepharose 4B (Pharmacia Fine Chemicals Inc., Piscataway, N. J.) in the presence of MgATP as described in detail in the legend to Fig. 1. Myosin ATPase activity appeared in a single peak at the elution volume (V_e) of rat muscle myosin (450,000 daltons) as illustrated in Fig. 1. As shown in Table II, 92% of myosin ATPase activity in the tissue-KT buffer extract was recovered as myosin by chromatography on Sepharose 4B. When the ATPase peak fraction shown in Fig. 1 was analyzed by SDS polyacrylamide gel electrophoresis (Fig. 2 A), a band with the mobility of rat muscle myosin heavy chain (200,000 daltons) was the principal constituent. The same material overloaded onto an 11% polyacrylamide gel (Fig. 2 E) demonstrated light chains of 14,000 and 19,000 daltons which correspond to the light chains of other myosins (8, 30).

The same myosin purification scheme (see Fig. 1 legend) was used for 1.9 g of principal pancreatic islets obtained from 32 kg of catfish and from 50 g of anterior and 20 g of posterior hog pituitary. Tissues were extracted with 2.5 vol of KT buffer. 100 U/ml of Trasylol was included with pituitary tissue buffers. In each case, a single peak of myosin ATPase activity was observed after Sepharose 4B chromatography at the molecular weight of native muscle myosin. Polyacrylamide gel electrophoresis was performed on each myosin ATPase peak (Fig. 2 B-D). The major constituent of each gel is a protein co-migrating with myosin heavy chain (200,000 daltons). In Fig. 3, purified catfish islet myosin is compared with the total islet tissue homogenate. Bands with the mobility of actin and myosin are prominent among catfish islet proteins (Fig. 3 A). In Fig. 3 C, rat skeletal muscle myosin was added to the islet myosin before electrophoresis. There is accentuation of myosin heavy chain and light chains 2 and 3 when compared with Fig. 3 B. Light chain 1 of muscle myosin has no counterpart in the catfish myosin.

The ATPase characteristics of the purified myosin peak fractions are presented in Table III. The enzymes have high ATPase activity in the presence of 0.6 M KCl and EDTA or calcium, but show little activity in the presence of magnesium, the pattern characteristic of smooth and skeletal muscle myosins. They have an average myosin (K^+-EDTA) ATPase specific activity of

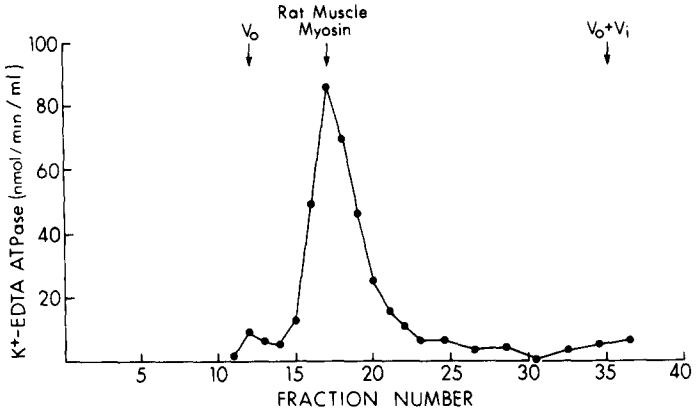

FIGURE 1 Sepharose 4B chromatography of hog parotid gland extract. 40 g of hog parotid glands was homogenized in 80 ml of KT buffer, and after 1 h at 0°C, the mixture was centrifuged at 12,000 *g* for 10 min and the supernate was fractionated with neutralized saturated ammonium sulfate in the presence of 2 mM ATP and 1 mM $MgCl_2$. The fraction precipitating between 30 and 60% of saturation was collected at 40,000 *g* for 30 min, taken up in 10 ml of KT buffer containing 0.5 mM MgATP (KT-AM buffer), clarified at 20,000 *g* for 10 min, and applied to a 2.5 × 107-cm Sepharose 4B column equilibrated with KT-AM. 15.2-ml fractions were collected. In a separate experiment, rat muscle myosin was eluted where indicated. V_o and $V_o + V_i$ indicate void volume and salt peak, respectively.

TABLE II

Purification of Hog Parotid Myosin

Fraction	Myosin ATPase	Recovery	Specific activity
	nmol/min	*%*	*nmol/min/mg protein*
KT extract	4,590	100.0	6.90
Ammonium sulfate fractions			
0–30%	310	6.8	1.57
30–60%	3,810	83.0	8.56
>60%	50	1.1	0.94
Sepharose 4B peak	4,200	91.5	396.0

361 nmol/min per mg which is similar to the value of 520 nmol/min per mg smooth muscle myosin, but much less than the 2,130 nmol/min per mg for skeletal muscle myosin. Likewise, the ratio of EDTA ATPase to calcium ATPase is 1.63 for secretory tissue myosin, resembling the 1.37 value of uterine muscle myosin, but much less than the 6.25 value of skeletal muscle myosin.

Hog anterior pituitary myosin could not be consistently prepared in the fashion described above unless the protease inhibitor Trasylol, 100 U/ml, was present in the buffers. The Sepharose 4B chromatography profiles of the myosin preparations shown in Fig. 4, demonstrate that in the absence of Trasylol the recovered ATPase had a distribution coefficient $(K_d)^1$ of 0.84 which is

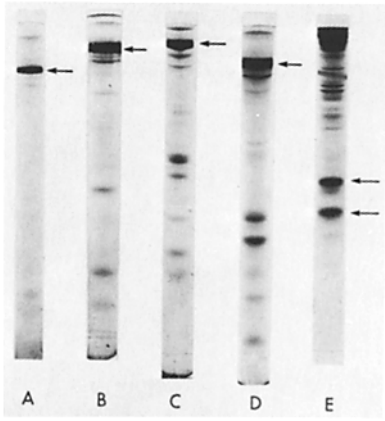

FIGURE 2 Polyacrylamide gels in 0.1% SDS of Sepharose 4B myosin ATPase chromatography peaks. All samples were centrifuged at 105,000 *g* for 90 min in KT-AM buffer before preparation for electrophoresis. (A) Hog parotid myosin, from Fig. 1 peak, 30 μg applied to a 5% polyacrylamide gel. (B) Catfish islet myosin, 25 μg applied to a 6% polyacrylamide gel. (C) Anterior pituitary myosin, 15 μg applied to a 6% polyacrylamide gel. (D) Posterior pituitary myosin, 25 μg applied to a 6% polyacrylamide gel. (E) same as A, except that 40 μg of hog parotid myosin was applied to an 11% polyacrylamide gel. Arrows in gels A-D refer to bands shown to co-migrate with muscle myosin heavy chain. Since gels were made and run at different times, migration of proteins differs. Arrows in E refer to light chain components of 14,000 and 19,000 daltons.

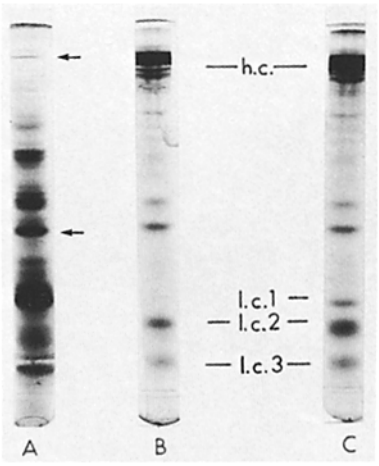

FIGURE 3 6% polyacrylamide in 0.1% SDS gel electrophoresis of catfish pancreatic islet proteins. (A) Total catfish pancreatic islet protein, 66 μg. Arrows denote bands with the mobility of muscle myosin (top) and actin. (B) Catfish islet myosin, 25 μg. (C) Catfish islet myosin, 25 μg, plus rat skeletal muscle myosin, 15 μg. h.c. and l.c. denote myosin heavy and light chains, respectively.

TABLE III

Purified Myosins

Tissue	ATPase activity			
	EDTA	Ca^{2+}	Mg^{2+}	EDTA/Ca^{2+}
	nmol/min/mg myosin protein			
Catfish	390	222	47	1.76
Hog parotid	396	203	32	1.95
Hog anterior pituitary	269	188	26	1.43
Hog posterior pituitary	388	285	15	1.36
Mouse uterus	520	380	30	1.37
Rat skeletal muscle	2,130	341	21	6.25

Myosins were purified by Sepharose 4B column chromatography followed by centrifugation at 105,000 *g* for 90 min in a type 40 rotor. ATPase was measured in a 2.2-ml reaction volume containing 0.6 M KCl, 10 mM imidazole (pH 7.0), 2 mM ATP, either 2 mM EDTA, 10 mM $CaCl_2$ or 5 mM $MgCl_2$, and ~35 μg of myosin over 30–60 min.

greater than that of whole myosin (K_d = 0.23). When 50 U/ml of Trasylol was included during purification, 71% of the recovered ATPase had the elution volume of myosin, whereas when 100 U/ml of Trasylol was used, 91% of the recovered ATPase activity was eluted as myosin. This suggests that proteolysis may occur during the isolation of pituitary myosin and that this difficulty is overcome by Trasylol. Only the molecular weight of myosin was reduced; the total recovery of pituitary myosin ATPase activity was not diminished in the absence of Trasylol. When a single hog pituitary was extracted as in Table I with and without 100 U/ml of Trasylol, the myosin ATPase activity was 0.317 ± 0.02 (mean ± SEM) and 0.323 ± 0.05 nmol/min per mg, respectively, for triplicate analyses.

Quantitation of Myosin in Secretory and Nonsecretory Tissue

Myosin was purified from the tissues listed in Table IV. The amount of myosin in a tissue was estimated from ATPase data as described in the

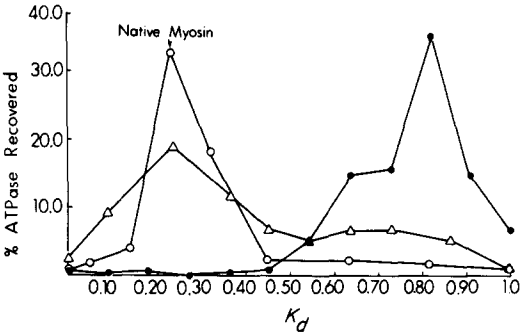

FIGURE 4 Effect of Trasylol on the elution of pituitary myosin ATPase. Hog pituitaries were extracted and ammonium sulfate-fractionated as described in the legend to Fig. 1 before chromatography on Sepharose 4B. Trasylol was included in the preparations at 0 (●), 50 (△), and 100 U/ml (○). Myosin (K^+-EDTA) ATPase was measured in each fraction and plotted as percent of the ATPase recovered from the column.

TABLE IV

Tissue Myosin Content

	(mg myosin × 100)/(mg cell protein*)	(mg myosin)/(g wet tissue)
Catfish islet	0.97	0.76
Hog parotid	0.65	0.29
Hog anterior pituitary	0.36	0.58
Hog posterior pituitary	0.21	0.17
Hog thoracic aorta	0.10	0.18
Mouse uterus	0.63	0.60

The amount of myosin was calculated as the myosin (K^+-EDTA) ATPase present in a 0.6-M KCl and 15-mM Tris-Cl (pH 7.0) tissue extract (nanograms of P_i per milligram of cell protein per minute) divided by the specific activity of the purified myosin after chromatography over Sepharose 4B (nanograms of P_i per milligram of myosin per minute).

* Protein not soluble in 1 N NaOH is excluded.

legend. Myosin constituted 0.2–0.97% of secretory tissue protein. The amount of myosin by this measure or expressed as milligrams of myosin per gram of wet tissue was similar in secretory and smooth muscle tissues such as aorta or uterus.

Binding of Pituitary Myosin to Actin

Binding was measured by determining the myosin ATPase remaining in the supernatant fraction after centrifuging pituitary myosin with muscle actin at 100,000 *g* (Table V). Under these conditions, actin readily sediments but myosin does not unless it is bound to actin. Pituitary myosin was found to bind rabbit skeletal muscle actin in the absence but not in the presence of ATP and $MgCl_2$.

TABLE V

Binding of Anterior Pituitary Myosin to Rabbit Muscle Actin

Sample	Supernatant myosin ATPase
	nmol/min/ml
Myosin	4.4
Myosin + actin	0.1
Myosin + actin + MgATP	4.3
Myosin + MgATP	3.8

Samples of pituitary myosin prepared in the presence of 100 U/ml of Trasylol and rabbit skeletal muscle actin were dialyzed against KT buffer to remove ATP. They were then mixed in a final volume of 0.5 ml of KT at a final concentration of 80 μg/ml myosin, 0.29 mg/ml actin, and, where indicated, 0.5 mM ATP and 0.5 mM $MgCl_2$. They were centrifuged at 105,000 *g* for 90 min to sediment the actin, and the supernate was then dialyzed against three exchanges of KT. Myosin ATPase was measured in the dialyzed supernate.

Activation of Secretory Tissue Myosin by Actin

Despite binding, secretory myosin ATPase was not significantly activated by skeletal muscle actins (Table VI). Preparation of pituitary myosin with 100 U/ml of Trasylol did not increase the degree of actin activation. Actins and myosins from different species interacted well, suggesting that species differences are of secondary importance (Table VI). The failure of rat muscle actin to activate hog pituitary myosin is more significant in light of the fact that rat muscle actin activated hog muscle myosin 17-fold.

Immunologic Studies

Hog parotid myosin was tested against antibodies to rabbit muscle myosin, mouse uterine myosin, and mouse L-cell fibroblast myosin (35) in Ouchterlony plates (Fig. 5). No precipitin line was seen with the muscle myosin antibody, but precipitin lines were seen with smooth muscle and fibroblast myosin antibody, indicating that the salivary gland myosin shares antigenic determinants with smooth muscle and fibroblast myosin, but not to a readily measurable extent with skeletal muscle myosin.

Solubility of Pituitary Myosin

Myosin from both muscle and nonmuscle sources was quantitatively extractable into buffers containing 0.6 M KCl. When either rat muscle (20 ml/g) or hog pituitary (3 ml/g) was homoge-

TABLE VI

Activation of Secretory Myosin ATPase by Muscle Actins

Myosin		Actin			ATPase		
Source	μg/ml	Source	μg/ml	KCl	−Actin	+Actin	(+Actin)/(−Actin)
				mM/liter	*nmol/min/mg myosin*		
Catfish islets	36	Rat	180	10	6.7	7.0	1.04
Hog anterior pituitary	23	Rat	193	10	16.7	20.5	1.23
Hog anterior pituitary	60	Rabbit	1,500	0	25.3	12.4	0.49
Human platelet	23	Rat	195	60	8.4	65.5	7.8
Hog skeletal muscle	89	Rat	193	17	9.8	168	17.1
Rat skeletal muscle	24	Rat	166	10	18.2	538	29.6
Rabbit skeletal muscle	60	Rabbit	1,500	0	63.0	412	6.54

Purified myosin and actin were assayed for ATPase activity in 10 mM imidazole (pH 7.0), 2.7 mM $MgCl_2$, 1 mM ATP, and small amounts of KCl as indicated. Assays were carried out at 37°C for 30–75 min in 0.1–2.2-ml volumes. Actins were prepared from skeletal muscle (see Materials and Methods).

nized and extracted three times with KT buffer. the first extract contained 92 and 99% of the total extractable myosin ATPase. respectively. This is in agreement with previously published data for tissue culture cells (22). However. pituitary myosin was much more soluble in low ionic strength buffers than skeletal muscle myosin. The extractability of muscle and pituitary myosin into hypertonic. isotonic. and hypotonic buffers is shown in Table VII. For purposes of comparison. the theoretical amount of myosin extractable from the tissue was taken as that amount extractable into 0.6 M KCl buffer (buffer 1). Pituitary myosin was 99% extractable into a buffer of physiologic ionic strength (buffer 2) and 47% extractable into a very hypotonic buffer useful for preparation of cell membranes (buffer 3). Muscle myosin. on the other hand. was only 0.3% extractable into the physiologic ionic strength buffer and could not be detected in the hypotonic buffer. The solubility of muscle myosin in buffer 2 was 4 μg/ml whereas that of pituitary myosin was in excess of 65 μg/ml. based on calculations of known specific ATPase activities of the myosins.

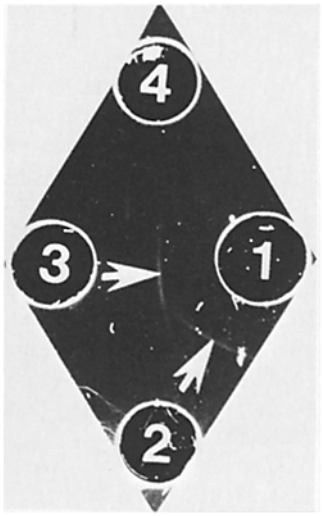

FIGURE 5 Ouchterlony immunodiffusion. Contents of the wells was as follows: (1) 20 μl of hog parotid myosin. 2.8 mg/ml. (2) 20 μl of goat anti-L-cell mouse fibroblast myosin serum. (3) 20 μl of goat anti-mouse uterine myosin serum. (4) 20 μl of goat anti-rabbit skeletal muscle myosin serum. Antibodies were prepared as described (35).

The solubility of pituitary myosin was also demonstrated by cell fractionation techniques. Table VIII shows that. under the conditions described. ~50% of the myosin ATPase activity was soluble at 105.000 *g*. suggesting that pituitary myosin is a predominantly cytoplasmic cell constituent. A small amount of myosin (0.6% of the total) was found associated with the purified secretory granule fraction and the secretory granule membranes.

DISCUSSION

The unique ATPase of myosin. that expressed in 0.6 M potassium chloride and 2 mM EDTA (21. 28). has been used to purify myosins from hog parotid. hog pituitary. and the principal pancreatic islet of the channel catfish. Secretory tissues were found to be enriched in this enzyme activity (Table I). Rat pancreatic islets. for example. contained 4.5 times the amount in rat liver. The large quantity of stored hormone should. if anything. have reduced the specific activity (nanomoles per minute per milligram) of myosin present in secretory tissues. Although the high ionic strength K^+-EDTA ATPase assay is obviously not conducted

TABLE VII

Extractability of Myosins

Buffer	Myosin ATPase			
	Muscle		Anterior pituitary	
	nmol/min/ml	*% of total*	*nmol/min/ml*	*% of total*
(1) KT Buffer	2,792	100	17.6	100
(2) ST Buffer, 2 mM DTT	8.3	0.3	17.5	99
(3) 10 mM Tris acetate (pH 6.5), 3 mM $MgCl_2$, 2 mM DTT	0.34	<0.01	8.3	47

Tissues, 0.15 g of rat hindlimb muscle or 1.0 g of hog anterior pituitary, were homogenized in 3.0 ml of buffer. After 1 h at 4°C, the homogenate was centrifuged at 105,000 *g* for 90 min and the supernates were dialyzed against KT buffer. Myosin ATPase (see Materials and Methods) in the dialysate was divided by 3 to give nanomoles per minute ATPase per milliliter of extracting buffer. The ATPase measured in buffer 1 was taken as 100%.

TABLE VIII

Pituitary Fractionation

Fraction	Myosin ATPase	Specific activity
	nmol/min	*nmol/min/mg*
Homogenate	6,450	0.94
	6,190	1.10
3,000-*g* pellet	650	0.43
	510	0.30
41,000-*g* pellet	400	0.27
	264	0.22
105,000-*g* pellet	340	1.41
	320	1.02
105,000-*g* supernate	3,360	2.89
	3,000	1.93
Secretory granules	29	0.21
	47	0.34
Secretory granule Membrane	24 18	0.60 0.55

50 g of hog anterior pituitary gland was homogenized in 100 ml of 0.3 M sucrose containing 50 mM Tris-Cl (pH 7.4) and fractionated as described in Materials and Methods. Each fraction was analyzed for protein content and then extracted for myosin ATPase activity with KT buffer. Two preparations were studied.

under physiological conditions, it may still contain useful information. For example, it has been demonstrated that the speed of muscle shortening correlated very well with the amount of high ionic strength K^+-EDTA ATPase in different skeletal muscles (5).

Secretory myosins were purified by extraction into 0.6 M KCl buffer, ammonium sulfate fractionation, and Sepharose 4B chromatography. Secretory tissue myosins had myosin ATPase activity (Table III), native molecular weight (450,000, Fig. 1), SDS subunit molecular weight (Fig. 2), and actin binding properties (Table V) very similar to those of smooth muscle myosin. The two myosins also shared antigenic determinants as seen in the cross-reactivity of hog parotid gland myosin with antibody to mouse uterus myosin and fibroblast myosin, but not to skeletal muscle myosin (Fig. 5). Secretory myosin might account for a portion of the actomyosinlike ATPase described previously in adrenal gland by Poisner (26, 27).

Myosin extracted from secretory tissues could not be accounted for by myosin in vessel smooth muscle cells present in these tissues. As noted in Table IV, the myosin content of aorta was about the same as that of secretory tissues. Mouse uterus was also similar in content of myosin to some secretory tissues. The highly vascular liver contained relatively little myosin ATPase (Table I). Finally, myosin has been purified directly from cloned GH_3 cells, a differentiated pituitary line capable of hormone secretion (21).

Two major problems were encountered in preparing secretory myosins. First, Trasylol-sensitive proteases destroyed several preparations of myosin during purification from pituitary tissue (Fig. 4). The protease activity in pituitary tissue has been well studied (11). Myosin-cleaving proteases have been described recently in platelets (1) and liver chromatin (10). In the last case, myosin could not be extracted intact without the inclusion of 8 mM diisopropyl fluorophosphate. Second, secretory myosins were soluble and could not be reliably collected by methods employing precipitation at low ionic strength, even if the pH was adjusted to 6.4 and if Mg^{2+} was present. This is in accord with published results for fibroblast myosin (3).

Secretory tissue myosins were readily soluble in buffers of physiologic ionic strength (Table VIII). During cell fractionation, ~50% of the recovered ATPase activity was found unassociated with particulate organelles in the 105,000-*g* supernate. The soluble nature of secretory tissue myosin under several buffer conditions (Table VII) suggests that, if filamentous myosin structures similar to the thick filaments of muscle exist in secretory cells, they must be easily disrupted. Myosin filaments (thick filaments) have been described infrequently in nonmuscle tissues, although the demonstration of such filaments even in smooth muscle requires considerable expertise (31). It should be pointed out that even the relatively soluble fibroblast myosin can form filaments in vitro (3).

Berl et al. (6) have demonstrated that a brain myosin-like substance is found in neurosecretory synaptic vesicles. Although these vesicles are not necessarily analogous to the larger secretory granules of pituitary, we noted that secretory granules and granule membranes had some myosin K^+-EDTA ATPase activity, even after extensive purification employing buffers in which myosin is soluble. It is possible that secretory granule membranes in vivo contain myosin as has been demonstrated for platelet (7) and fibroblast (35) plasma membranes.

Secretory tissue myosin was able to bind normally to rabbit skeletal muscle actin and be released by MgATP (Table V). Despite this, no significant activation of the low ionic strength

myosin ATPase by actin was observed under conditions where skeletal muscle and platelet myosins demonstrated normal activation (Table VI). Inasmuch as actin-activated myosin ATPase is dependent upon the phosphorylation of a myosin light chain in several tissues (2, 13), the myosins as isolated may be nonphosphorylated. It has been suggested that acute phosphorylation regulates actomyosin contraction in smooth muscle (13). Another explanation for lack of actin activation of myosin ATPase is absence of an actomyosin cofactor such as that found in rabbit alveolar macrophages (32).

The present study does not allow any conclusions to be drawn as to the function of myosin in secretory tissues. However, a variety of alterations in contractile proteins might accompany hormone secretion. In rat pancreatic islets, the total and polymerized content of tubulin increased with chronic stimulation of insulin secretion (24). In platelets, a myosin light chain could be phosphorylated in vitro by a specific kinase, and the resulting phosphomyosin had increased actin-activated ATPase activity (2). In vivo, myosin phosphorylation occurred as a result of thrombin treatment of platelets (9), a secretory stimulus. Our aim is to define a role for myosin in the secretory process.

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