PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 150, 299–302 (1975)

Actomyosin Isolated from Bovine Tracheal Smooth Muscle[1] (39023)

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It is now thought that the native actomyosin of skeletal muscle, i.e., actomyosin whose ATPase activity is either stimulated by calcium or inhibited by EGTA[2], is composed of four distinct proteins: actin, myosin, troponin and tropomyosin. Troponin, in combination with tropomyosin, inhibits the ATPase activity of actomyosin. This inhibition is reversed by the binding of calcium to troponin. The addition of EGTA, a chelating agent with high affinity for the calcium ion, results in inhibition of the ATPase reaction by reversing the above process (1). Even though the presence of actomyosin in uterus (2, 3) and carotid (4) smooth muscle has been reported, much less is known regarding the nature and control of the contractile components of smooth muscle than is known about the contractile components of skeletal muscle. Calcium stimulation of smooth muscle actomyosin has been difficult to demonstrate and inconsistently found (3–9). Recent reports have demonstrated that chicken gizzard smooth muscle preparations lack troponin (5, 9) and it has been suggested that the regulatory system of smooth muscle differs from that of skeletal muscle (5, 9). This report describes a "classical" preparation of actomyosin from bovine tracheal smooth muscle. Activities of this protein were not stimulated by calcium or inhibited by EGTA. No evidence of a troponin–tropomyosin regulatory system could be demonstrated in this tissue.

Materials and methods. Bovine tracheal actomyosin was prepared essentially as described by Sparrow *et al.* (7) for bovine carotid smooth muscle. Trachea were removed from cows within one half hour of death and transported to the laboratory in a cold physiological salt solution (PSS) (7). The smooth muscle was stripped from the trachea, cut into small pieces and placed in PSS minus calcium. Approximately 500 g of smooth muscle was homogenized (at 4°) in 2.5 ml of homogenization media per gram of tissue using a Sorvall Omni-Mixer at full speed (30 sec of homogenization at 30 sec intervals for a total homogenization time of 2 min). The homogenization media contained: 80 m*M* KCl; 4 m*M* EGTA; 4 m*M* $MgCl_2$; 20 m*M* MOPS pH 7.0; 0.5 m*M* DTT; 4 m*M* ATP (sodium salt). The homogenate was extracted by stirring overnight at 4°. The following morning the pH was readjusted to 7.0 and ATP was added to give an additional 1 m*M*. The homogenate was then stirred for 10 min at 4°. The insoluble material was removed by centrifugation at 12,000*g* for 1.5 hr. The supernatant was dialysed overnight against two 12-liter vol of dialysis buffer A (125 m*M* KCl 4.4 m*M* MOPS pH 7.0; 0.5 m*M* DTT, 0.2 m*M* EGTA) at 4°. The precipitate was collected by centrifugation at 10,000*g* for 30 min and then redissolved in 200 ml of a solution containing 600 m*M* KCl, 1 m*M* ATP pH 7.0. The solution was then centrifuged at 12,000*g* for 1.5 hr and the supernatant dialysed overnight against two 12 liter vol of buffer A. The precipitate was then collected, redissolved, and dialysed as above. The resulting precipitate was collected by centrifugation and was washed with buffer B (50 m*M* KCl, 0.5 m*M* DTT, 1 m*M* $MgCl_2$, 1 m*M* Na Azide, 8 m*M* MOPS pH 7); centrifuged for 30 min at 10,000*g* and then resuspended and stored in buffer B, at 4°.

Bovine skeletal muscle troponin and tropomyosin were prepared by the method of Greaser and Gergely (10) and shown to be active when added to desensitized bovine skeletal muscle actomyosin.

ATPase activity was determined by placing

[1] This work was supported by NIH Grant No. HL-14964.

[2] Abbreviations used: EGTA, Ethyleneglycol-*bis*-(β-Amino-Ethylether) *N,N'*-Tetra-Acetic acid; MOPS, Morpholinopropane sulfonic acid; DTT, Dithiothreitol.

0.1–0.4 mg of actomyosin protein in one of the following buffer solutions: A, 17.5 m*M* MOPS pH 7.0; B, 17.5 m*M* MOPS pH 7.0, 10 m*M* $MgCl_2$, plus 1 m*M* EGTA; or C, 17.5 m*M* MOPS pH 7.0 10 m*M* $MgCl_2$ plus 1 m*M* $CaCl_2$. The reaction was started by the addition of ATP (2.2 m*M* final concentration). Final volume was 1.0 ml. The reaction took place at 25° and was terminated by the addition of 0.3 ml of 30% TCA. After centrifugation at 250*g* for 10 min the inorganic phosphate released was determined by the method of Rockstein and Herron (11).

Superprecipitation was measured in the same solutions as used for the determination of ATPase. To a final volume of 3.0 ml, 2.4 mg of actomyosin was added in graduated centrifuge tubes. After 10 min at room temperature the tubes were centrifuged for 20 sec at 1400*g* in a clinical centrifuge and the amount of precipitate was visually determined.

Protein was determined by the method of Lowry *et al.*, (12) using bovine serum albumin as standards. All water used in these experiments was first deionized and then distilled. Dialysis bags were boiled in 0.5 m*M*-$NaHCO_3$ and 1 m*M* EGTA to assure minimal contamination by calcium.

Results and discussion. The data in this report are the first concerning the presence of actomyosin in tracheal smooth muscle. The protein obtained by the "classical" procedures outlined above meets four of the necessary criteria for it to be classified as actomyosin (Fig. 1–3). (a) It is extracted at a low ionic strength in the presence of ATP; (b) it is soluble at high ionic strength (600 m*M* KCl) and insoluble at low ionic strength (125 m*M* KCl); (c) it has ATPase activity and (d) it demonstrates superprecipitation on the addition of ATP. ATPase activity was low, (relative to skeletal muscle actomyosin), as has been reported for actomyosin from other smooth muscle. However, tracheal smooth muscle actomyosin differs from other smooth muscle actomyosins in that it possesses significant ATPase activity without the addition of divalent cations (Figs. 1 and 2). The tracheal smooth muscle ATPase activity (Figs. 1 and 2) as well as the superprecipitation reaction (Fig. 3) was not influenced by

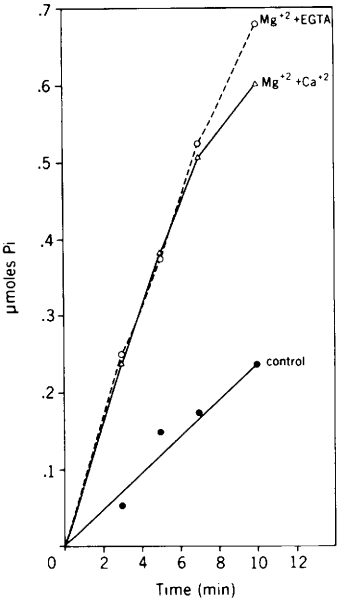

FIG. 1. ATPase activity of tracheal smooth muscle actomyosin. Values shown are the mean of six determinations.

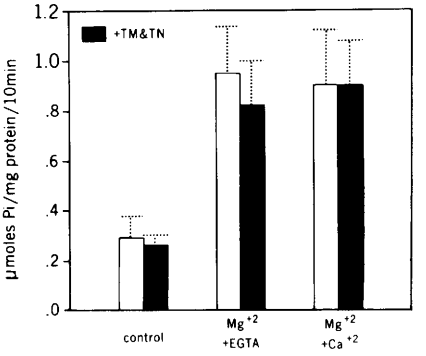

FIG. 2. Specific ATPase activity of tracheal smooth muscle actomyosin with and without the addition of bovine skeletal muscle troponin (TN) and tropomyosin (TM). The reaction mixture contained 0.31 mg of tracheal smooth muscle actomyosin and where indicated 0.50 mg of bovine skeletal muscle tropomyosin and 1.1 mg of bovine skeletal muscle troponin. Values shown are the mean of five determinations ± SD.

the presence of either calcium or EGTA. This lack of response was not reversed by the addition of active bovine skeletal muscle troponin–tropomyosin (Figs. 2 and 3) or by extracts from tracheal smooth muscle similarly prepared and possibly containing smooth muscle troponin–tropomyosin (data

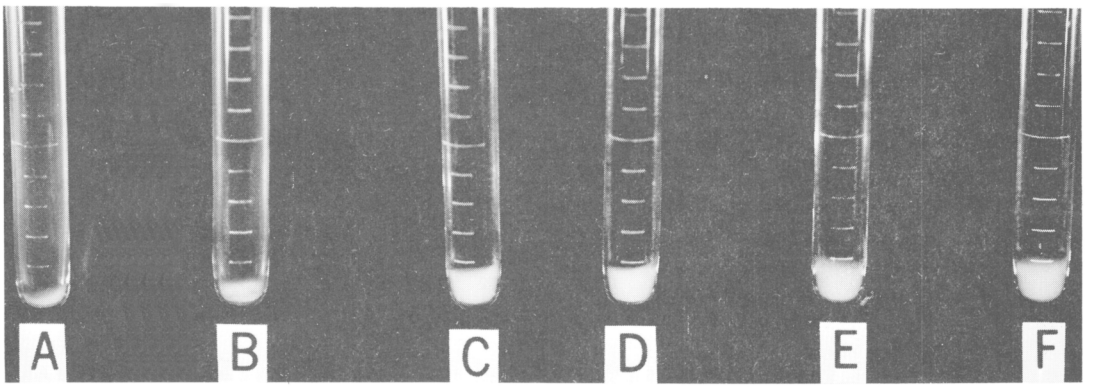

FIG. 3. The superprecipitation reaction of tracheal smooth muscle actomyosin (24 mg) with and without the addition of bovine skeletal muscle troponin (0.8 mg) and tropomyosin (0.3 mg). (A) No ATP; (B) ATP; (C) ATP + Mg + Ca; (D) ATP + Mg + EGTA; (E and F) Same as C and D plus active bovine skeletal troponin and tropomyosin.

not shown). It is important to note that essentially similar results (ATPase of similar activity and lacking an effect of either calcium or EGTA) were obtained by us using the tracheal smooth muscle actomyosin extracted by the high ionic strength procedure of Mallin (13) and the low ionic strength procedure of Filo *et al.* (4); and the actomyosin extracted from glycerinated tracheal smooth muscle by the method of Shibata *et al.* (14).

Others have found calcium stimulation or EGTA inhibition of the activity of smooth muscle actomyosin difficult to demonstrate. Murphy *et al.* (6) were unable to demonstrate a calcium ion dependence for the ATPase activity from bovine carotid smooth muscle. However, Sparrow *et al.*, report that they were able to demonstrate calcium stimulated ATPase activity by actomyosin derived from the same tissue (7). They concluded that the addition of EGTA and DTT to all extraction solutions, and an ionic strength of 0.12 for the extraction solution instead of 0.05 were the significant differences. The procedures used in this report incorporated their suggestions, yet the actomyosin isolated still lacked sensitivity to calcium or EGTA. Carsten has prepared actomyosin from uterine smooth muscle using techniques similar to those used in this report (3). She found that the uterine actomyosin was highly unstable and had low ATPase activity. This is in agreement with our findings for tracheal smooth muscle actomyosin. She also found that the EGTA sensitivity of the uterine actomyosin was relatively low and could not be demonstrated in all preparations. However, she could demonstrate troponin–tropomyosinlike activity in extracts of uterine smooth muscle. This finding was not duplicated by us using tracheal smooth muscle, or by Bremel (5) and Driska and Hartshone (9) using smooth muscle from chicken gizzard.

In a recent report Driska and Hartshone (9) suggest that treatment of the chick gizzard smooth muscle preparation with the nonionic detergent, Triton X-100, was necessary before a calcium-sensitive actomyosin could be isolated. The mechanism of action of the detergent in unmasking of the calcium-sensitivity is not known. We plan to study the effect of the detergent on the activity of the bovine tracheal smooth muscle actomyosin.

We conclude that tracheal smooth muscle does contain actomyosin. However we have not been able to demonstrate that the various activities of this protein when extracted by "classical" procedures, are calcium stimulated or EGTA inhibited and have found no evidence for troponin–tropomyosin regulation in this tissue. The data in this report support the suggestion (5, 9) that the regulatory system of vertebrate smooth muscle differs from that of skeletal muscle.

Summary. A contractile protein (actomyosin) was isolated from bovine tracheal smooth muscle by the use of "classical" pro-

cedures. The protein was considered to be actomyosin because it demonstrated: ATPase activity; superprecipitation upon the addition of ATP; and the solubility and extraction characteristics of actomyosin. The ATPase and superprecipitation reactions were not inhibited by EGTA, and did not require calcium. Lack of an effect of either calcium or EGTA could not be reversed by the addition of active bovine skeletal muscle troponin and tropomyosin. No troponin–tropomyosin like activities could be demonstrated in various tracheal muscle fractions.

The author would like to thank the Pepper Packing Company of Denver for their generosity and cooperation in supplying bovine trachea. The skillful technical assistance of T. Meyer, W. Penberthy and R. Jorgensen is acknowledged with gratitude.

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Received April 11, 1975. P.S.E.B.M. 1975, Vol. 150