ACTOMYOSIN-LIKE PROTEIN ISOLATED FROM THE ADRENAL MEDULLA

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1. Introduction

The events involved in the process by which the adrenal medulla secretes catecholamines have been called 'stimulus-secretion coupling' because of their similarity to 'excitation-contraction coupling' in muscle [1]. Like muscle contraction the secretory process, in addition to Ca^{2+}, requires energy, perhaps in the form of ATP [2]. Furthermore, we have also shown that ATP in the presence of Mg^{2+} induces conformational changes in isolated chromaffin granules leading to the release of soluble granule components (catecholamines, ATP and soluble protein) [3,4]. These, and other observations [5,6] have suggested that the response to ATP is contractile in nature and that it might be mediated through contractile elements present either in the granules or in the chromaffin cell [3,7]. These suggestions have been strengthened by the recent observation of interactions between rabbit actin, chicken myosin and chromaffin granule membranes [8]. Furthermore, the presence in the brain of an actomyosin-like protein has been already described [9]. Therefore, it was decided to investigate further the presence of an actomyosin-like protein in the adrenal medulla and we now describe the isolation of a Mg^{2+}- or Ca^{2+}-activated adenosine triphosphatase (ATPase) from bovine adrenal medulla.

2. Materials and methods

Bovine adrenal glands obtained from a slaughterhouse were kept on ice and the medullae were separated from the cortices.

2.1. *Extraction of proteins*

a) *Procedure 1:* Each medulla was homogenized in 3 volumes of Weber–Edsall solution (0.6 M KCl, in a bicarbonate buffer, pH 8.2) in a waring blender for 20 min at 4°C. The homogenate was kept at 4°C for 16–18 hr and then centrifuged at 60 000 *g* for 60 min. The supernatant thus obtained was diluted with distilled water in order to lower the concentration of KCl to 0.1 M. The pH of this solution was adjusted to 6.3 by the addition of 0.125 M acetate buffer, pH 4.9. The preparation was allowed to stand on ice for 60 min and a precipitate was separated by centrifugation at 20 000 *g* for 10 min. The sediment thus obtained was suspended in 0.6 M KCl, containing 0.05 M Tris–HCl buffer, pH 7.2 Distilled water was added again to the preparation in order to dilute the KCl to 0.1 M. The preparation was allowed to stand on ice for 60 min and the precipitate which developed was separated by centrifugation at the speed indicated above. This resuspension and precipitation cycle was repeated once more. This method of isolation yielded 3.8 ± 0.65 mg protein per g of wet medulla (n = 17).

b) *Procedure 2:* Fifty grams of bovine skeletal muscle and 100 g of adrenal medullary tissue were used for the preparation of myosin according to the method described by Perry [10]. The final adrenal and muscle preparations were obtained in 0.5 M KCl [10]. Distilled water was added to the preparations in order to lower the concentration of KCl to 0.014 M. Both preparations were centrifuged at 30 000 *g* for 10 min and the pellets were resuspended in 0.1 M sodium phosphate buffer (pH 7.2) containing 1% sodium dodecylsulfate (SDS) and 1% mercaptoethanol. The preparations were digested for 1 hr at 45°C and samples were taken for electrophoresis and column chromatography.

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2.2. *Polyacrylamide gel electrophoresis*

SDS disc electrophoresis was performed by the method of Weber and Osborn [11]. Six percent gels were used and the applied current was of 8 mA per tube for 5 h. The gels were stained with Coomassie brilliant blue and destaining was performed electrophoretically [11]. In order to determine the molecular weight of the different protein bands of the preparations standards of different molecular weight were also run.

2.3. *Column chromatography*

Samples of muscle and adrenal preparations were applied simultaneously to two identical Sephadex G-200 columns (2.6 × 60 cm). The chromatographies were carried out following the method of Starr and Offer [12], a technique which was described for the resolution of muscle myosin in different components. The columns were eluted with 0.1 M sodium phosphate buffer (pH 7.2) containing 0.1% SDS.

3. Results and discussion

The protein extracted by the procedure 1 exhibited ATPase activity and, as indicated in table 1, the ATPase was activated to a greater extent by Ca^{2+} than by Mg^{2+}. This appears to be different to the cationic activation of that of skeletal muscle [13], but seems similar to that of smooth muscle [14]. Actomyosin and myosin have ATPase activity and both are activated by Ca^{2+} [13]. If the ionic strength of the incubation medium is low, actomyosin predominates, whereas at high ionic strength in the presence of ATP, actomyosin dissociates into actin and myosin and the ATP-splitting activity has the characteristic of the myosin ATPase, i.e., inhibition by Mg^{2+} [13]. The protein isolated from adrenal medulla appears to behave somewhat similarly to contractile protein from muscle since the ATPase activity of the preparation was greater in a medium of low ionic strength (0.06 M KCl). Furthermore, there was no additive effect when both Mg^{2+} and Ca^{2+} were present in the incubation medium (table 1), as if these ions were activating the same enzyme.

Szent-Györgyi discovered that, under certain conditions (0.1 M KCl, 5 mM $MgCl_2$), the reaction of actomyosin with ATP results in its coagulation and rapid precipitation [15]. This phenomenon called superprecipitation has been accepted as an unorganized analogue of muscle contraction [13,15]. The protein isolated from the adrenal medulla showed such characteristics (fig.1). The addition of Mg^{2+} (3×10^{-3} M) to the preparation did not change its absorbance, but when ATP (2.5×10^{-4} M) was added in the presence of Mg^{2+} there was a significant increase in the turbidity of the preparation (fig.1). This was followed by the formation of a dense precipitate that settled at the bottom of the tube. The increase in turbidity upon the addition of ATP was inversely proportional to the protein concentration in the solution (fig.1A), presumably because precipitation occurred faster with the increase in the protein concentration (fig.1A). When the protein and ATP concentrations were constant, the changes in turbidity and the precipitation time were independent of Mg^{2+} concentrations (10^{-8} M to 10^{-3} M), but when the Mg^{2+} and protein concentrations were constant, the increase in turbidity

Table 1
Effect of KCl concentration on the Mg^{2+}- or Ca^{2+}-activated ATPase isolated from adrenal medulla

Activating Ion	ATPase activity (μmoles Pi/mg protein/h)	
	0.06 M KCl	0.6 M KCl
Mg^{2+}	2.27 ± 0.04** (22)*	1.20 ± 0.03 (11)
Ca^{2+}	4.16 ± 0.07 (18)	1.82 ± 0.07 (12)
$Mg^{2+} + Ca^{2+}$	2.08 ± 0.08 (8)	1.30 ± 0.02 (10)

* Number of experiments between parenthesis
** Mean ± S.E.M.

The ATPase activity was determined at 37°C 30 min after adding aliquots (0.7 mg protein) to centrifuge tubes containing 1 ml of the following solution: 0.03 M *N*-tris (hydroxymethyl)methyl-2-aminoethane sulfonic acid (TES) beffer, pH 7.0; 0.001 M ouabain; and either 0.06 or 0.6 M KCl. Mg^{2+} and/or Ca^{2+} were added to the preparation at the concentration of 2 mM [γ-^{32}] ATP (specific activity: 0.18 μCi/μmole) was added to the medium to give a concentration of 1 mM. The incubation was stopped by adding ice-cold trichloroacetic acid (10% final concentration). The tubes were coolded in a water bath for a few minutes and the ^{32}P released during the incubation was measured as previously described [18,19]. The results were expressed in micromoles of Pi per mg of protein per hour, calculated on the basis of the specific activity of the added [γ-^{32}P] ATP [18,19].

was higher and the precipitation time shorter with increasing ATP concentration from 10^{-5} to 10^{-3} M (fig.1B).

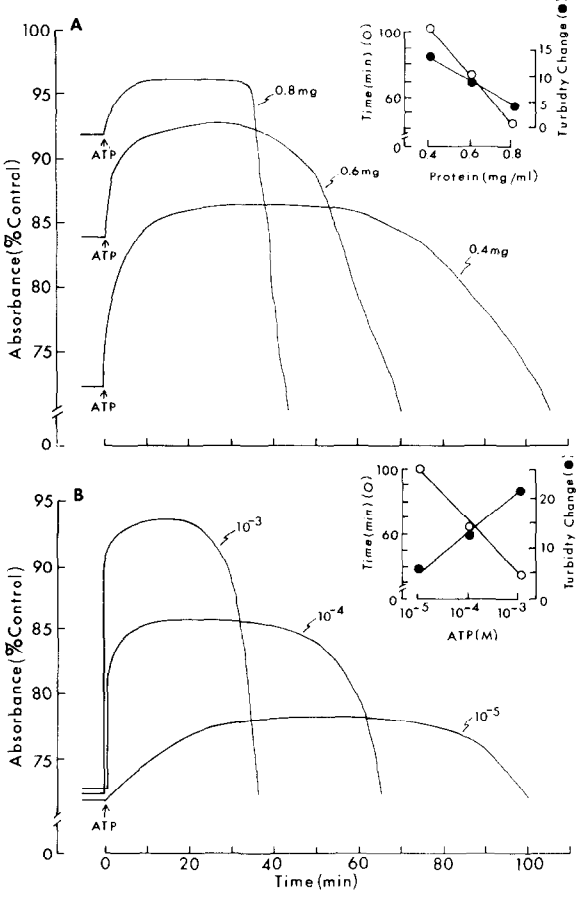

Fig.1. Effects of protein and ATP concentrations on the superprecipitation of the actomyosin-like protein isolated from the adrenal medulla. (A) Aliquots of protein (0.4, 0.6 and 0.8 mg/ml) were added to cells containing 2 ml of incubation medium (0.1 M KCl and 0.005 M Tris–HCl buffer, pH 7.2). Five minutes later Mg^{2+} (3×10^{-3} M) and ATP (2.5×10^{-4} M) were added to the medium and the changes in the absorbance of the preparation were monitored (540 nm) and recorded in a Zeiss PMQII spectrophotometer. The inset shows the relationship between the changes in turbidity or time of precipitation and the concentration of protein in the incubation medium. (B) Aliquots of protein (0.4 mg/ml) were added to three cells each containing 2 ml of the incubation medium. The inset shows the relationship between the changes in turbidity or time of precipitation and the concentration of ATP in the incubation medium. Other conditions as in (A).

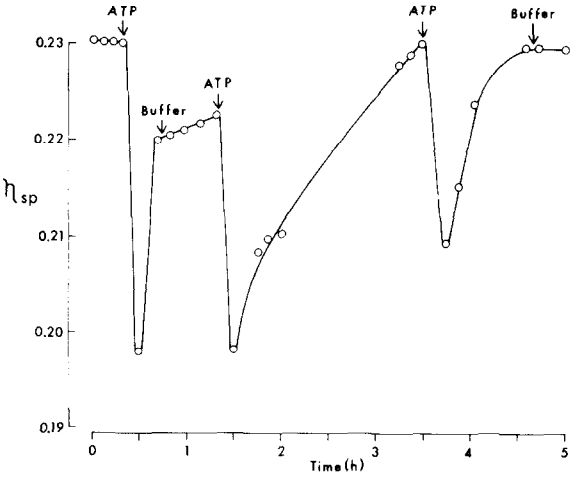

Fig.2. Effect of ATP on the specific viscosity of the actomyosin-like protein isolated from the adrenal medulla. The measurements were made at 22°C in a Cannon-Fenske dilution viscometer size 50, containing 6.3 ml of the following solution: 0.6 M KCl and 0.005 M Tris–HCl, pH 7.2. The protein concentration was of 2.2 mg/ml and neutralized ATP of buffer (50 μl) were added at the time indicated by arrows. Two 25 μl aliquots of a 100 mM ATP solution were added to the medium (first and third arrows from left to right). The final ATP concentration in the medium was of 4×10^{-4} M. The last addition of ATP (fourth arrow) was of 50 μl of the same solution; the final ATP concentration being of 8×10^{-4} M. The specific viscosity (η_{sp}) was calculated by the following formula: $\eta_{sp} = (OT_e - OT_b)/(OT_b)$. The data was calculated in terms of outflow times of buffer (OT_b) and extract (OT_e).

The data in table 1 showing a decrease in the ATPase activity of the preparation in a medium of high ionic strength would seem to indicate a dissociation of the actomyosin-like protein of the adrenal medulla into two components, an actin- and a myosin-like component. To investigate this further the viscosity changes of a solution of this adrenal protein were determined. Figure 2 shows that there was a rapid decrease in the viscosity of the preparation upon the addition of small amounts of ATP ($4–8 \times 10^{-4}$M).

This effect of ATP could be obtained repeatedly with the same preparation but the addition of the same volume of Tris-HCl buffer had no effect on the specific viscosity of the preparation (fig.2). The change in the specific viscosity of the preparation upon the addition of ATP, although significant, was much smaller than that reported for muscle proteins [13].

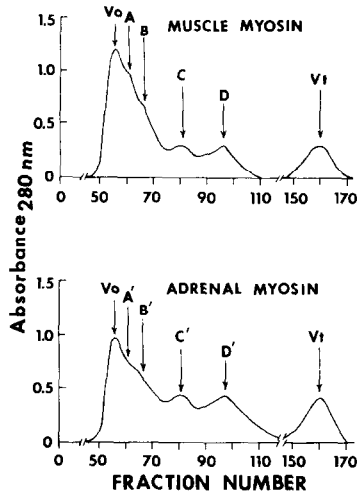

Fig.3. Separation of the polypeptide components of muscle and adrenal myosin by gel filtration in SDS. Samples (24 mg protein each) of denature (SDS + mercaptoethanol) muscle and adrenal myosin were applied to 2 columns (2.6 × 60 cm) of Sephadex G-200. The columns were equilibrated and eluted with 0.1 M sodium phosphate buffer (pH 7.2) containing 0.1% SDS. The chromatophies were carried out at 25°C and the columns were perfused with a flow rate of 17 ml/hr. Polyacrylamide disc gel electrophoresis was performed with fractions A, B, C, D, A′, B′, C′ and D′ (see fig.4).

This is probably due to the fact that our preparation was not as pure as were the muscle preparations.

To obtain more information about the adrenal proteins and in order to compare these proteins to those found in muscle, SDS polyacrylamide gel electrophoresis and gel filtration chromatography were carried out on the adrenal and on the muscle preparations obtained by procedure 2. The electrophoretic patterns show that with the exception of two bands (mol. wt. 79 000 and 60 000 in the adrenal preparation) there was a good correlation between both preparations in the band mobilities (fig.4). The myosin band of the adrenal preparation was smaller than that present in muscle preparations. This is not surprising, since it has been pointed out that actin is the main component of the contractile proteins in non-muscle cells [16]. The skeletal muscle preparations show bands which correspond to heavy myosin (mol. wt. 220 000), to actin (mol. wt. 46 000), to tropomyosin (mol. wt. 41 000) and to the light chains of myosin (mol. wt. 27 000, 22 000 and 16 000). These different muscle

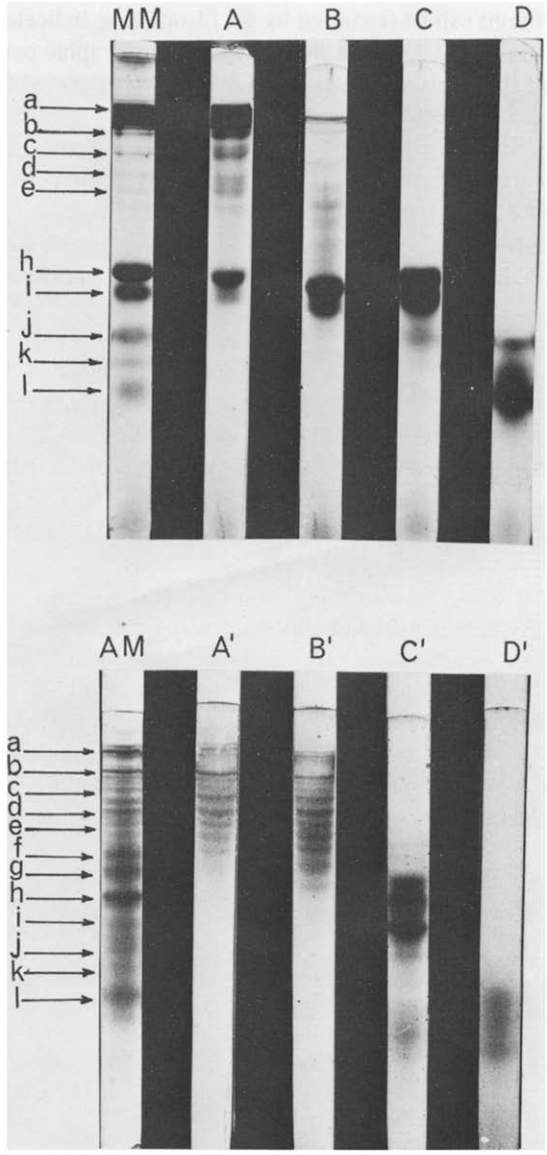

Fig.4. SDS polyacrylamide gel electrophoresis of muscle and adrenal myosin polypeptides before and after separation by Sephadex G-200 chromatography. Muscle (MM) and adrenal (AM) myosin preparations isolated by the procedure described by Perry [10] were subjected to SDS polyacrylamine electrophoresis following the method of Weber & Osborn [11]. Electrophoresis was also carried out with samples taken from fractions eluted from the Sephadex G-200 columns (see fig.3) at A, B, C, D, A′, B′, C′ and D′. The direction of migration was from top to bottom, which was the anode. The molecular weights of the bands as determined by the use of protein standards were 220 000; 180 000; 155 000; 135 000; 112 000; 79 000; 60 000; 46 000; 41 000; 27 000; 22 000 and 16 000 for bands a, b, c, d, e, f, g, h, i, j, k and l respectively.

proteins can be separated by gel filtration as indicated in figs.3 and 4, which show the chromotographic patterns and the electrophoresis of the different chromatography peaks respectively.

Similar chromatography and electrophoretic patterns were observed for the adrenal meddullary proteins (figs.3 and 4). Whether these adrenal proteins of electrophoretic mobilities equivalent to muscle proteins are structurally and functionally related to the muscle contractile proteins should be determined in future experiments.

The present results and the preliminary observation of Poisner [7] seem to indicate the presence in the adrenal medulla of an actomyosin-like protein which, like muscle actomyosin, has a) ATPase activity and colloidal properties which are affected by Ca^{2+}, by KCl and by Mg-ATP concentrations; and b) similar gel filtration and electrophoretic patterns.

It is also worthwhile to point out that we have previously published data on the solubilization and partial characterization of a Mg^{2+}-dependent ATPase of the membranes of chromaffin granules [18]. Whether this protein, which is also phosphorylated by ATP [18,19] is a component of the actomyosin-like protein described here is now under investigation in our laboratory.

Acknowledgements

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