ELECTROPHORETIC INVESTIGATION OF MUSCLE EXTRACTS OF LOW IONIC STRENGTH

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The composition of protein subunits extracted from muscle homogenates of various types by salt media of low ionic strength was studied by electrophoresis in polyacrylamide gel with sodium dodecyl sulfate. Extracts from smooth muscles contained a premyosin subunit with a molecular weight of 230,000, the heavy chain of myosin, actin, and certain other proteins. Extracts of low ionic strength from smooth muscles possessed Mg- and Ca-activated ATPase activity. A premyosin subunit also was found in extracts of low ionic strengths from homogenates of skeletal muscles. It is postulated that the premyosin subunit is a component of the enzyme system responsible for the ATPase properties of extracts of low ionic strengths from homogenates of different types of muscle.

KEY WORDS: ATPase; myosin; actin; electrophoresis of proteins; smooth and skeletal muscles

Myosins isolated from different types of muscles are known to differ both in their ATPase activity and in certain other properties, especially the number of light chains. Unlike skeletal muscles and myocardium, the myosin and actomyosin of smooth muscles are extracted by salt media of low ionic strengths and have much lower ATPase activity [1, 3, 4].

In this investigation extracts of low ionic strengths from muscles of different types were compared by electrophoresis in polyacrylamide gel (PAG), using sodium dodecyl sulfate (SDS).

EXPERIMENTAL METHOD

Smooth muscles from the stomach and an artery, "slow" red skeletal muscles from the diaphragm and soleus, and "fast" white muscle from the psoas, obtained from rabbits, and muscles from the bovine diaphragm and uterus were used. The rabbit was killed, the stomach was immediately removed and quickly freed from food residues and rinsed in 0.05 M Tris-HCl buffer, pH 7.8, to inactivate pepsin; the muscle tissue was then freed from mucosa and connective tissue. The skeletal muscles were removed at the same time. Bovine muscles were isolated after slaughter of the animal and were kept in ice. The purified muscles were put thorugh a mincer or cut up finely with scissors, then homogenized for 1 min in a homogenizer with Teflon pestle and washed 6 or 7 times with water or 5 or 6 times with 0.15 M NaCl and twice with water to remove blood. The washed homogenate was treated with 3 volumes of the extracting buffer solution of the following composition: 0.05 M KCl, 25 mM Tris-HCl, pH 7.2, 0.2 mM ATP, and 2 mM β-mercaptoethanol. Extraction was carried out consecutively for 3-40 min, 4 h, and 16-18 h with constant stirring. A fresh portion of cold buffer solution was used for each extraction. The extracts were centrifuged the first time at 18,000g (30 min) and the second at 40,000g (2 h). After each centrifugation approximately two-thirds of the volume of fluid in the centrifuge tube was carefully decanted and the rest was discarded. The resulting extracts were concentrated by dialysis against dry Sephadex G-200 but the extracts from the gastric muscles were used without concentration. In some cases (the diaphragm) the extracts were dialyzed after concentration on Sephadex against 0.05 M KCl, and the slight turbidity which formed was removed by centrifugation.

All operations for treatment of the muscles and preparation of the extracts were performed at 0-4°C with double-distilled water.

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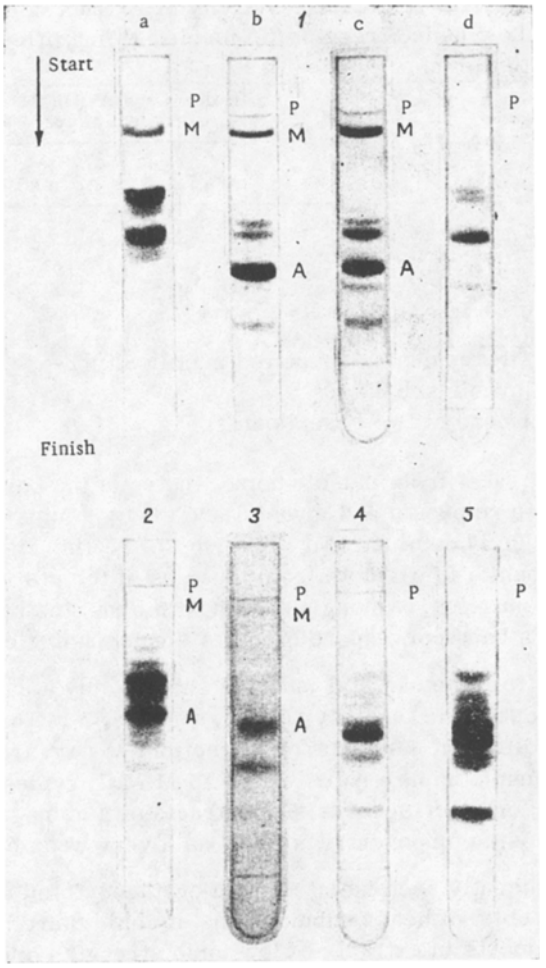

Fig. 1. Electrophoresis of extracts of low ionic strengths (4% PAG): 1) smooth muscles of rabbit stomach: fundus – extraction for 10 min (a) and 5 h (b), pylorus – extraction for 5 h (c) and after 3rd change of buffer solution (d); 2) smooth muscles of uterus, extraction for 12 h; 3) smooth muscles of artery, extraction for 12 h; 4) slow skeletal muscle (diaphragm); 5) slow skeletal muscle (soleus); P) premyosin subunit; M) heavy chain of myosin; A) proteins with electrophoretic mobility of actin.

Electrophoresis in PAG using SDS was carried out as described by Weber and Osborn [7], using mainly 4% gels [5]. The strength of the current was 8 mA per tube and the duration of electrophoresis about 4 h. Proteins were taken simultaneously from different types of muscles and, after treatment with mercaptoethanol and SDS, they were applied to the gels in 3 different concentrations. Protein was determined by Lowry's method, using serum albumin as the standard; ATPase activity was determined at 25°C by the usual method, based on the increase in inorganic phosphate.

EXPERIMENTAL RESULTS

Electrophoresis of extracts of low ionic strengths from smooth muscles from the fundal and pyloric portions of the rabbit stomach in 4% PAG revealed considerable amounts of two subunits with low electrophoretic mobility and several fast-moving subunits (Fig. 1, strips 1). Of the two subunits with low electrophoretic mobility one was the heavy chain of myosin, with mol. wt. $2.0 \cdot 10^5$–$2.1 \cdot 10^5$, the other was premyosin with mol. wt. about $2.3 \cdot 10^5$. The ratio between the premyosin and myosin heavy subunits in the extracts from the gastric muscles differed depending on the duration of extraction. In 3- to 10-min extracts mainly the heavy subunit of myosin was present, whereas after longer extraction, besides the heavy chain of myosin, considerable amounts of the premyosin subunit also were extracted, especially after the homogenate had been washed 5 or 6 times with 0.15 M NaCl (Fig. 1, strips 1). The subunit with mol. wt. $2.3 \cdot 10^5$ was also found recently in the composition of purified smooth-muscle actomyosin [6]. During electrophoresis in 10% PAG both heavy subunits gave a common band at the start.

TABLE 1. ATPase Activity of Extracts of Low Ionic Strengths (in nmoles P_i/mg protein/min)

Test object	Hydrolysis of ATP in presence of undetermined ions	
	Mg^{2+} (1,0 mM)	Ca^{2+} (10 mM)
Rabbit stomach	11,5±5,8	2,1±0,3
Part of rabbit stomach*:		
Fundus	4,0†	—
Pylorus	4,8†	—
Diaphragm	4,5†	2,65†

*Heavy chains consisted mainly of premyosin subunits.

†Mean of two experiments.

During electrophoresis of extracts from muscle homogenates of the bovine uterus and rabbit artery double bands of the heavy subunits (premyosin and myosin) and also a series of bands of proteins with higher electrophoretic mobility were regularly obtained (Fig. 1, strip 2). Examination of the gels after electrophoresis shows that the content of heavy subunits in extracts from muscles of the uterus and artery was relatively lower than in extracts from the stomach muscles. Among subunits with high electrophoretic mobility, in extracts from all types of smooth muscles a band corresponding in its electrophoretic mobility to actin also was found.

Electrophoresis of extracts from the skeletal muscles showed only a narrow band of the premyosin heavy subunits as a protein of low electrophoretic mobility (Fig. 1, strips 4, 5). This band was constantly present in extracts of the slow muscles with different durations of extraction. As regards extracts from the fast psoas muscle, repeated washing of the muscle homogenates with 0.15 M NaCl evidently removed practically all of the premyosin component. Meanwhile, on electrophoresis of extracts of a homogenate of muscles previously washed only with water, the premyosin component was found as a very weak band.

A myosin-like ATPase has recently been isolated from erythrocyte membranes and, during analogous electrophoresis, it behaved as a doublet of heavy subunits [2]. It could therefore be suggested that the heavy subunit found in the present experiments in extracts of low ionic strengths originated from erythrocyte membranes. However, a control experiment with blood showed that with the procedure of washing the muscle homogenates that was used, all the blood was removed from them.

Extracts of low ionic strengths from homogenates of all types of muscles exhibited ATPase properties. ATPase activity was activated by Mg^{2+} and less strongly by Ca^{2+} and was inhibited by ethyleneglycoltetraacetate.

ATPase activity (Table 1) was highest in extracts from smooth muscles of the rabbit stomach. Besides other proteins, an actomyosin of smooth-muscle type or, to use the terminology of Laszt and Hamoir [3], tonoactomyosin, is extracted along with other proteins by salt media of low ionic strengths from smooth muscles [3, 4], and for that reason the ATPase properties of the extracts might have been connected with that protein. It is an interesting fact that extracts from the smooth muscles of the stomach, in which the heavy chains are represented almost exclusively by the premyosin component (Fig. 1, strips 1) also possessed ATPase activity.

Much lower ATPase activity was found in low ionic strength extracts from skeletal muscles. The magnitude of this activity correlated with the quantitative content of the premyosin subunit in these extracts and was higher in the slow red muscles. Removal of the premyosin subunit as a result of prolonged washing of the psoas muscle with 0.15 M NaCl was accompanied by loss of its ATPase activity.

It can be concluded from these results that the so-called tonoactomyosin of smooth muscles is a complex protein system which includes at least two types of heavy subunits: myosin and premyosin, as well as, evidently, several proteins with lower molecular weight. The premyosin subunit also was found in extracts of low ionic strength from homogenates of slow (diaphragm, soleus muscle) and fast (psoas) skeletal muscles, but whereas its contribution is higher in the red muscles it is negligibly small, as the investigation showed, in white muscles.

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ELECTROPHORETIC INVESTIGATION OF NUCLEAR MEMBRANE RIBONUCLEASES OF RAT LIVER AND HEPATOMA-27 CELLS

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Preparations of the nuclear membranes were obtained from purified nuclei of rat liver and hepatoma-27 cells, and from them enzyme-containing extracts of acid-soluble proteins were then prepared. The protein extracts were subjected to disk electrophoresis in 15% polyacrylamide gel. Ribonucleases (RNases) which are constituents of the acid-soluble proteins of the nuclear membranes of normal liver were found to be present as several different components which differed in their electrophoretic mobility and in several physicochemical properties from crystalline bovine RNase and the RNase of nuclear chromatin.

KEY WORDS: electrophoresis; polyacrylamide gel; nuclear membranes; ribonucleases; rat liver; hepatoma-27

Besides the many ribonucleases (RNases) contained in the cytoplasm and its organoids, a high proportion of RNase activity is also contained in the cell nuclei [8, 16]. Despite the comparatively low relative proportion of the nuclear depolymerases, their role in the metabolism of the cellular RNA is evidently very important [17]. By now several RNases hydrolyzing different types of RNA have been found in the protein composition of the cell nuclei, and in their properties these enzymes differ significantly from the cytoplasmic enzymes [13]. Since polyribonucleotide transport from nucleus into cytoplasm probably takes place through the nuclear pores [1, 2], the nuclear membranes must play an important role in the enzymic degradation of RNA.

It has been shown [6, 7] that the layers of the nuclear membranes possess high RNase activity. There is some evidence that the nuclear membranes contain not one, but several enzymes degrading RNA [7]. The study of this problem could be important for a detailed explanation of the mechanism of transport and the realization of the genetic potentials of the cell nucleus. After fractionation in polyacrylamide gel (PAG) the activity of the RNases is still preserved and can be detected by electrophoresis [9, 18].

The object of the present investigation was an electrophoretic study of RNases found in the cell membranes of rat liver and hepatoma-27.

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