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Biophysics

The Influence of Smitin on Fermentative Activity of Actomyosin in Different Area Conditions

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ABSTRACT. The influence of smitin (C-titin) on Mg^{2+}-activated ATPase activity of chicken smooth muscle (stomach) actomyosin in different area conditions (ionic strength, pH, different concentrations of smitin) was studied. It was shown that smitin, likewise titin, causes the increasing of Mg^{2+}-activated ATPase activity of actomyosin. Mg^{2+}-activated ATPase activity in the presence of smitin has maximal value in 30mM KCl and minimal - in 100mM KCl. Mg^{2+}-activated ATPase activity of actomyosin in the presence of smitin reaches maximal value at pH 8 and at pH 9 it decreases. ATPase activity increases according to the growth of smitin concentration and is maximal when it makes up 40% of myosin by weight. Obtained results confirm that in smooth muscle smitin has the same effect on actomyosin ATPase activity as titin has on skeletal muscle ATPase activity. Smitin stipulates muscle elastic properties, on the one hand, and on the other hand it is the "scaffold" for the proteins participating in muscle contraction, forming the supermolecular complex with these proteins. *© 2014 Bull. Georg. Natl. Acad. Sci.*

Key words: *smitin, titin, Mg^{2+}-activated ATPase activity.*

In the late fifties of the past century, it was already well known that in muscle fibers there exist elastic components that differ from collagen fibers. These components were supposed to be responsible for their elastic properties, especially for passive stretch. In 1954 Natori [1] showed, that passive stretch develops while uncoated fibers stretched and after removing the stretching force they returned to the initial state. This fact indicates that myofibrils contain elastic components.

Up to 1976 the chemical nature of the substance responsible for intercellular continuity and elasticity was unknown. In 1967 the Hungarian scientist Guba [2] extracted the protein - fibrilin, that formed T-protofibrils. This fact unfortunately was forgotten. Later Japanese scientists Maruyama et al. [3] extracted elastic protein from myofibril that was responsible for continuity. He called that protein "connectin". Later Wang [4] began detailed biochemical study of connectin and showed that it was the mix of two different proteins with molecular masses 3000 kD and 800 kD. He named the big protein "titin", and the

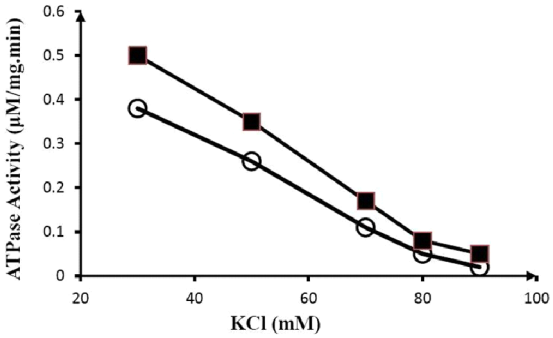

Fig. 1. Effects of smitin on Mg^{2+}-activated ATPase activity of actomyosin at various KCl concentrations. O – control, ^% –with smitin (20% of myosin by weight), myosin /actin 4:1, t = 30^0 C, 1mM $MgCl_2$, 1mM ATP, 0.05M Tris HCl, pH 8.

small one, which was difficult to reveal – "nebulin". These two proteins together compose 15% of myofibril proteins. The method of immunoelectron microscopy helped to localize these proteins in sarcomere. Titin molecule has the form of thread approximately with the length of 1.2 mM, and in skeletal and heart muscles it spans half a sarcomere (from M line up to Z disc). It connects the myosin end with Z disc and provides the tension transfer. Titin consists of 27 000 amino acids. It contains repetitive immunoglobulin (Ig G) sites consisting of 100 amino acids and unique fragment (PEVK) rich with proline, glutamine, valine and lysine.

Titin is extracted from chicken and rabbit skeletal muscles by Kimura and Maruyama [5], Wang [2,6], Trinic and collaborators [7,8]. It should be mentioned, that while titin purification with ammonium sulphate it irreversibly aggregated and it was impossible to obtain super pure titin specimen to study its physical-chemical properties. Using the Trinic method modified by us, we managed to obtain ultra -pure native titin and investigated its some hydrodynamic and physical-chemical properties [8-10].

In structures isolated from different organs of the same organisms (skeletal and thorax muscles, stomach, gizzard) and different organisms (rabbit, chicken, fish, amphibian) there are titin and titin-like macromolecules. As far back as 1977 Maruyama et al. [11] showed that smooth muscle contained titin-like high molecular protein, but it was not investigated at that time. Only in 2002 it was established that smooth muscle contained protein with 2000 KDa mass that was called smitin, further C-titin. In molecular morphology and localization in contractile apparatus it is very similar to titin and belongs to their group. Investigations showed that smitin and titin are different proteins, but they are encoded by the same gene and are distinct isoforms of differential splicing. Smitin is also supposed to play central role in myosin filament organization in contractile apparatus and other cells of smooth muscle *in vivo*.

Around today as opposed to titin there is scant information about smitin physical-chemical properties. Only its interaction with myosin [12] and a-actinin [13] is studied. Our collaborators [14] extracted smitin from chicken smooth muscle (stomach) and investigated its physical parameters. It is interesting to determine smitin role in smooth muscle tonic contraction, where as opposed to striated muscle, neither sarcomere is distinctly formed nor Z disc presents and consequently contraction degree is different. Hence, it is important to study smitin interaction with other proteins of smooth muscle and its function in it.

Kimura and Maruyama [15] investigated titin interaction with myosin, actin and actomyosin in low ionic strengths conditions. They also studied titin influence on actomyosin Mg^{2+}-activated ATPase activity in different KCl concentrations and at different pH.

The goal of present paper was to study smitin influence on actomyosin Mg^{2+}-activated ATPase activity in the terms of different ionic strength, different pH and different smitin concentrations and to show the resemblance and diversity with the corresponding data of titin obtained from striated muscle.

Materials and Methods

Smitin was isolated from chicken smooth muscle, particularly from stomach [16]. Myofibrils were obtained according to Wang [6]. Extract of myofibrils was applicated on the toyopearl 65 column [14], obtained fractions contained smitin, myosin and other proteins. Myosin fractions were collected and used

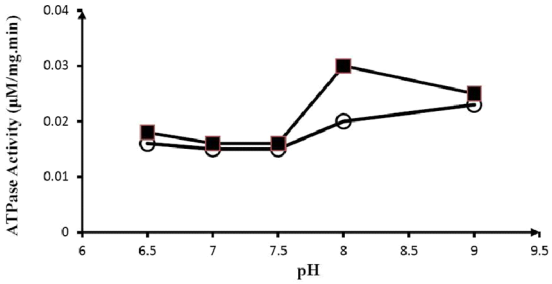

Fig. 2. Effects of smitin on Mg^{2+}-activated ATPase activityof actomyosin at various pH.
O – control, ˆ%–with smitin, 60mM KCl. Other conditions were the same as in Fig.1 except that pH varied as shown in the abscissa.

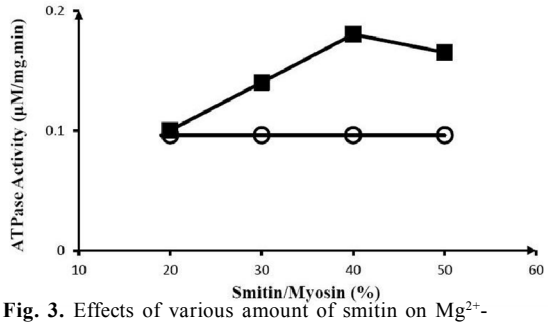

Fig. 3. Effects of various amount of smitin on Mg^{2+}-activated ATPase activity of actomyosin
O – control, ˆ% –with smitin, 30mM KCl. Other conditions were the same as in Fig.1 except that smitin amounts (%) varied as shown in the abscissa.

in experiments. Actin was extracted according to Straub. Reconstructed actomyosin was obtained by mixing of F-actin and smooth muscle myosin 1:4 correspondingly.

Protein concentrations were determined spectrophotometrically assuming extinction coefficients $El_{280}^{1img/ml}$ 0.57 for myosin and 1.09 for actin [17]. Smitin concentration was evaluated according to [18].

ATPase activity was evaluated according to the amount of inorganic phosphate split from ATP [19].

Results and Discussion

Fig.1. shows smitin influence on actomyosin Mg^{2+}-activated ATPase activity in presence of different concentrations of KCl. Smitin concentration was 20% of myosin by weight. As one can see, Mg^{2+}-activated ATPase activity of actomyosin in the presence of 1mM $MgCl_2$ and 1mM ATP reaches the maximum at 30mM KCl. Activity is too low at 100mM KCl and higher concentrations.

Smitin influence on actomyosin Mg^2 -activated ATPase activity in the presence of 60mM KCl, 1mM $MgCl_2$ and 1mM ATP at different pH has been studied. Fig. 2 shows that smitin maximal effect is detected at pH 8. ATPase activity is low at pH 6.5 and lower and at pH 9. In the mentioned experiments smitin concentration was 20% of myosin by weight.

Fig. 3 shows the influence of increasing concentrations of smitin on Mg^{2+}-activated ATPase activity of actomyosin. As one can see, the change of smitin concentration from 20% to 50% by weight of myosin increases ATPase activity which reaches maximum when smitin concentration is 40%. When smitin concentration is 50%, ATPase activity is less.

Obtained experimental results confirm that in smooth muscle smitin has the same function as titin has in skeletal muscle.

Table shows the influence of smitin on ATPase activity of smooth muscle actomyosin in the presence of Ca^{2+}. As we can, see smitin has the same effect on actomyosin ATPase activity as titin has on skeletal muscle ATPase activity [20]. In the presence of smitin (20%, 30%) actomyosin ATPase activity increases by10-30%, correspondingly, while the growth of skel-

Table. Smitin influence on actomyosin Mg^{2+}-activated ATPase activity (2mM $MgCl_2$, 0.1 mM $Ca^{2+}$and 1mM ATP, our data are compared with those for titin [20]

Ca^{2+} concentration	ATPase activity		
	Smooth and striated muscle actomyosin (control)	Striated muscle actomyosin + titin	Smooth muscle actomyosin + smitin (our data)
1. pCa =4,6	100%	159%,	110% (smitin20%)
2. pCa =7,5	100%	133%,	130% (smitin30%)

etal muscle ATPase activity in the presence of 20% titin is quite high - 60%.

Vikhliancev et al. [20] studied titin influence on skeletal muscle actomyosin ATPase activity in the presence of Ca^{2+} (pCa 4,6 and pCa 7,5). Why did they use Ca^{2+}, but reconstructed actomyosin does not contain troponin? May be the effect of Ca ions concentration is stipulated by Ca^{2+} binding with nontroponin sites. When pCa=4.6 the ATPase activity of rabbit skeletal muscle actomyosin in the presence of titin increases roughly by 60% (Table) [20], and in our case - in the presence of smitin (20% - 30% of myosin by weight) – by 10% -30%. So, we can suppose that high concentrations of Ca^{2+} and smitin effect nonactin sites of muscle.

Obtained results also verify that contractive process and fermentative activity in smooth muscle is lower than in skeletal muscle.

ბიოფიზიკა

სმიტინის გავლენა აქტომიოზინის ფერმენტულ აქტივობაზე სარეაქციო არის სხვადასხვა პირობებში

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შესწავლილია სმიტინის (C-ტიტინის) გავლენა ქათმის გლუვი კუნთის (კუჭი) აქტომიოზინის Mg^{2+}-აქტივირებად ATP-აზურ აქტივობაზე სარეაქციო არის სხვადასხვა პირობებში (იონური ძალა, pH, სმიტინის სხვადასხვა კონცენტრაცია). ნაჩვენებია, რომ სმიტინი ტიტინის მსგავსად იწვევს აქტომიოზინის Mg^{2+} -აქტივირებადი ATP-აზური აქტივობის ზრდას. Mg^{2+}-აქტივირებადი ATP-აზური აქტივობა სმიტინის თანაობისას მაქსიმალურია 30mM KCl –ის და მინიმალურია 100mM KCl –ის პირობებში. Mg^{2+} -აქტივირებადი ATP-აზური აქტივობა სმიტინის თანაობისას მაქსიმუმს აღწევს pH 8.0, ხოლო pH 9-ზე მცირდება. აქტივობა იზრდება სმიტინის კონცენტრაციის მატებისას და მაქსიმუმს აღწევს სმიტინის 40% კონცენტრაციის დროს (მიოზინის წონაზე გადაანგარიშებით). მიღებული შედეგები ადასტურებს, რომ გლუვ კუნთში სმიტინი იგივე ფუნქციას ასრულებს, როგორსაც ტიტინი – ჩონჩხის კუნთში. სმიტინი განაპირობებს ერთის მხრივ გლუვი კუნთის ელასტიურ თვისებებს, ხოლო მეორე მხრივ წარმოადგენს "ეშაფოტს" კუნთის შეკუმშვაში მონაწილე ცილებისათვის, წარმოქმნის რა სუპერმოლეკულურ კომპლექსს ამ ცილებთან.

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