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The isoforms of α-actin and myosin affect the Ca^{2+} regulation of the actin-myosin interaction in the heart

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ABSTRACT

Myocardium of mammals contains a wide range of isoforms of proteins that provides contractile function of the heart. These are two isoforms of ventricular and two of atrial myosin, α- and β-tropomyosin, and two isoforms of α-actin: cardiac and skeletal. We believe that the difference in the amino acid sequence of α-actin can affect the calcium regulation of the actin-myosin interaction. To test this hypothesis, we investigated effects of the isoforms of α-actin, cardiac and skeletal, and the isoforms of cardiac myosin on the calcium regulation of the actin-myosin interaction in an *in vitro* motility assay using reconstructed regulated thin filaments. The results show that isoforms of α-actin and the ratio of α/β-chains of Tpm differently affect the calcium regulation of the actin-myosin interaction in myocardium in dependence on cardiac myosin isoforms.

1. Introduction

Heart of mammals is the extremely specialized organ composed of four cameras (left and right ventricles and atria) that differ its structure and function. Ventricles and atria contain a range of the isoforms of contractile and regulatory proteins that determine its contractile function. In myocardium of mammals, there are two isoforms of myosin heavy chains (MHC): α and β [1]. In ventricle, together with ventricular isoforms of light chains (vLC), they form two isomyosins: V1 and V3, homodimers consisting of α- and β-MHC, respectively [2]. In atria, α- and β-MHC together with atrial LCs (aLC) form A1 (αα) and A2 (ββ) isomyosins [2]. The identity of amino acid sequence of α- and β-MHC is 93% [3]. The differences in the amino acid sequences of α- and β-MHC in the catalytic domain and in the 'lever arm' determine the difference in their mechanical and kinetic characteristics [3–6]. Unique properties of atrial myosin are specified by aLC [7,8].

There are also two isoforms of α-actin: cardiac and skeletal, encoded by ACTA1 and ACTC genes, respectively [9,10]. These two isoforms differ by only 4 of 375 amino acid residues, two of which are located in the myosin-binding site [11]. Expression of the α-actin isoforms is species specific. The heart of adult human contains around 20% skeletal α-actin [10]. It was found that in the fetal hearts, a content of skeletal α-actin is at least 50% but after birth, it greatly is reduced [10].

In cardiac muscle two tropomyosin (Tpm) isoforms are expressed, Tpm1.1 (α) and Tpm2.2 (β) [12] identical by 87% (39 amino acid substitutions) [13]. In the adult murine heart, the α-Tpm isoform constitutes up to 100% of the total Tpm. The adult human hearts predominantly express α-Tpm with 3–5% of β-Tpm [14]. During cardiogenesis β-Tpm is expressed at moderate levels [15]. There are functional differences between the two highly conserved Tpm isoforms of striated muscle [15–19].

In pathological states of myocardium, the isoform composition of sarcomeric proteins is changing. Izumo et al. [20] showed that at pressure overload of the rat heart, the expression of some proteins (β-chain of Tpm, skeletal α-actin, β-MHC) is increased. Study on mice with dilated cardiomyopathy mutant tropomyosin demonstrated increased expression of both skeletal α-actin and β-MHC [19]. Under heart pressure overload, a fraction of the skeletal isoform of α-actin increases [21–23].

The changes in the contractile apparatus of cardiomyocyte play important role in maintenance of myocardial contractile activity in pathology. The role of the cardiac myosin isoforms in cardiac contractility was intensively investigated but it is little known on the role of the α-actin isoforms. Hewett et al. [24] revealed that changes in the isoform composition of α-actin affect functioning of the heart muscle.

Previously, we investigated the characteristics of single actin-

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myosin interactions of cardiac and skeletal isoforms of α-actin with the isoforms of cardiac myosin using an optical trap technique and an *in vitro* motility assay and found that the isoforms of α-actin do not affect mechanics and kinetics of the interaction [8]. We believe that the α-actin isoforms may affect calcium regulation of the actin-myosin interaction.

Studies on mammalian hearts have demonstrated the importance of expression of contractile and regulatory protein isoforms during both ontogenesis and pathology. Studies that utilized whole heart or isolated preparations of myocardium cannot provide clear understanding of the role of each isoforms in cardiac contractility. An *in vitro* motility assay enables studying the actin-myosin interaction at the level of thin filament by combining different isoforms of regulatory and contractile proteins and thus analyzing their contribution into the interaction at molecular level. The aim of this work was to study effects of α-actin isoforms, and tropomyosin with different content of α- and β-chains on the calcium regulation of the actin-myosin interaction in the heart.

2. Materials and methods

All procedures involving animal care and handling were performed according to institutional guidelines set forth by Animal Care and Use Committee at the Institute of Immunology and Physiology of RAS and Directive 2010/63/EU of the European Parliament.

2.1. Preparation of proteins

Cardiac [10] and skeletal α-actins were prepared respectively from left ventricles and *m. psoas* of the euthyroid rabbits by standard procedure [25]. Cardiac troponin from the left ventricles of the euthyroid rabbits was isolated as described by Potter [26]. To reveal the importance of Tpm β-chain for the calcium regulation of actin-myosin interaction in myocardium we used Tpm with a high β-chain content from *psoas* muscle. Tropomyosin was extracted from the left ventricles and *psoas* muscle of the euthyroid rabbits [27]. The α- and β-chain content of Tpm was determined using 8% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) with 4 M urea [28]. Gels were stained with Coomassie Brilliant Blue R250. Cardiac Tpm was α-chain homodimer, αα-Tpm, and skeletal Tpm contained 60% α- and 40% β-chains, αβ-Tpm (Fig. 1A).

V1 and V3 isomyosins were obtained from hyper- and hypothyroid rabbits, respectively [5]. Atrial myosin was obtained from atria of the euthyroid rabbits. Myosin was extracted according to standard technique [29]. Composition of MHC was determined using SDS-PAGE [30]. Myosin from hyperthyroid rabbits contained 90% α-MHC and 10% β-MHC, myosin from hypothyroid rabbits contained 10% and 90% α- and β-MHC (Fig. 1B), and vLCs. Myosin from atria (A1) contained 100% α-MHC (Fig. 1B) and aLCs. Before experiment, 'dead' myosin molecules were removed by ultracentrifugation with F-actin and ATP [5]. Myosin concentration was measured with Bradford assay (Sigma-Aldrich Co. LLC.).

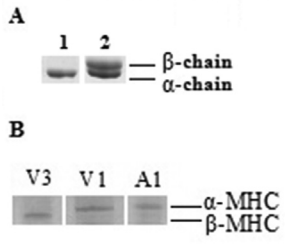

Fig. 1. (A) Gel electrophoresis of tropomyosin from left ventricles (1) and *m. psoas* (2) of the rabbits. (B) The isoform composition of myosin heavy chains (MHC) of atrial myosin from the euthyroid rabbits and ventricle myosin from the hypothyroid (V3) and hyperthyroid (V1) rabbits.

The reconstitution of regulated thin filaments from F-actin, troponin and Tpm was done according to Gordon et al. [31] in AB buffer containing 25 mM KCl, 25 mM imidazole, 4 mM $MgCl_2$, 1 mM EGTA, and 10 mM DTT, pH 7.5.

2.2. In vitro motility assay

Protocol of the experiment in the *in vitro* motility assay and measurement of the sliding velocity of thin filaments using the GMimPro software were as described earlier [32,33]. The experiments were done at 30 °C.

The '*p*Ca–velocity' experiments were carried out three times with each of the isoforms and the means of individual experiments were fitted to the Hill equation: $V = V_{max}\ (1 + 10^{h(pCa\text{-}pCa50)})^{-1}$, where V and V_{max} are velocity and maximal velocity obtained at saturating calcium concentration, respectively, pCa_{50} (calcium sensitivity) is *p*Ca at which half maximal velocity is achieved, and h is the Hill coefficient. All values are expressed as mean ± S.D. All comparisons were performed by the Mann-Whitney U test ($p < 0.05$).

3. Results

We studied effects of α-actin isoforms on the calcium regulation of the actin-myosin interaction in the *in vitro* motility assay. For this, the sliding velocity of thin filaments reconstructed from either skeletal or cardiac isoform of α-actin, tropomyosin with different content of α- and β-chains, and troponin over cardiac isomyosins at different calcium concentration was measured.

When α-actin isoforms were used with α-MHC isomyosins, i.e. V1 and A1, they affected pCa_{50} of the '*p*Ca–velocity' relationship. The calcium sensitivity of the sliding velocity of thin filaments containing cardiac α-actin with αα-Tpm was significantly higher than with αβ-Tpm (Fig. 2A; Table 1). However, with thin filaments containing skeletal α-actin the calcium sensitivity of the velocity over V1 and A1 isomyosins did not depend on ratio of Tpm α/β-chains (Fig. 2B; Table 2).

The calcium sensitivity of the filament sliding velocity over V3 myosin did not depend on the isoforms of α-actin. (Fig. 2; Tables 1 and 2). The presence of the Tpm β-chain markedly increased pCa_{50} of the velocity over V3 myosin. Note that the presence of β-chain of Tpm exerted the opposite effect on the calcium sensitivity of the '*p*Ca–velocity' relationship of myosin with α-MHC (V1 and A1) and β-MHC (V3).

The Hill coefficient of the '*p*Ca–velocity' relationship of V1 and V3 isomyosins did not depend on the α-actin isoforms and the ratio of α/β-chains of Tpm (Fig. 2; Tables 1 and 2). The Hill coefficient for A1 myosin was lower with thin filaments containing cardiac α-actin and αα-Tpm as compared to other thin filament compositions (Fig. 2; Tables 1 and 2).

The maximal sliding velocity of thin filaments containing both cardiac and skeletal α-actin over V1 and A1 myosin, but not over V3 isomyosin, depended on the ratio of Tpm α/β-chains (Tables 1 and 2). The maximal sliding velocity was higher with thin filaments containing αα-Tpm.

The calcium sensitivity of the '*p*Ca–velocity' relationship of V1 and V3 was higher as compared to A1 with all compositions of thin filaments used (Fig. 2; Tables 1 and 2). This result is in accordance with early data obtained on the preparations of myocardium [34].

Thus, the calcium regulation of the actin-myosin interaction in