ISSN 0006-2979, Biochemistry (Moscow), 2008, Vol. 73, No. 2, pp. 178-184. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © L. V. Nikitina, G. V. Kopylova, D. V. Shchepkin, L. B. Katsnelson, 2008, published in Biokhimiya, 2008, Vol. 73, No. 2, pp. 219-227.
Originally published in Biochemistry (Moscow) On-Line Papers in Press, as Manuscript BM07-244, December 16, 2007.

Study of the Interaction between Rabbit Cardiac Contractile and Regulatory Proteins. An *in vitro* Motility Assay

L. V. Nikitina*, G. V. Kopylova, D. V. Shchepkin, and L. B. Katsnelson

Institute of Immunology and Physiology, Ural Division of the Russian Academy of Sciences, ul. Pervomaiskaya 91, 620041 Ekaterinburg, Russia; fax: (343) 374-0070; E-mail: l.nikitina@iip.uran.ru

Received July 13, 2007
Revision received August 22, 2007

Abstract—A series of experiments was performed in an *in vitro* motility assay with reconstructed thin filaments to obtain pCa–force relationships for cardiac isomyosins V1 and V3. Two concentrations of each isomyosin (200 and 300 μg/ml) on the surface of a flow cell were tested. Isometric force was estimated as the amount of actin-binding protein, α-actinin, stopping thin filament movement. It was found that the amount of α-actinin stopping the movement at saturating calcium concentration for V3 was twice higher than for V1 at both concentrations of isoforms. Hill coefficients of cooperativity (*h*) were determined for pCa–force relationships. The value of *h* did not differ significantly for isoforms at 300 μg/ml of protein (*h* was 1.56 for V1 and 1.54 for V3). However, the Hill coefficient was higher for V3 isoform at 200 μg/ml (*h* = 2.00 and 1.76 for V3 and V1, respectively). Importantly, the Hill coefficient increased for both isoenzymes when their concentrations were decreased. The connection between Hill coefficient and cooperative interactions between cardiac contractile and regulatory proteins is analyzed in detail.

DOI: 10.1134/S0006297908020090

Key words: cardiac isomyosins, cooperativity between contractile and regulatory proteins

Force generation in muscle is achieved by using the energy of ATP hydrolysis by cross-bridges formed by myosin heads and actin filaments. There are two types of myosin heavy chains in mammalian heart—α and β [1]. Myosin isoforms V1 and V3 are homodimers of α- and β-heavy chains, respectively.

It is known that expression of myosin isoforms V1 and V3 is different in cardiomyocytes from different layers of the cardiac ventricle wall [2-4]. Isomyosin V1 is located mostly in subepicardium, while subendocardium contains more isomyosin V3 [3].

Expression of these myosin isoforms depends upon species, age, and hormonal state of the animal [5]. Isomyosin V1 is predominant in small adult mammals (mice, rats), while isoform V3 is found in hearts of larger mammals including humans. Changes in the expression of these isoforms are also associated with cardiac pathologies. Thus, most cardiac pathologies caused by heart pressure or volume overload show a shift from V1 to V3 expression [6, 7].

Functional properties of heart isomyosins have been most studied on rodent cardiac tissues. For example, it was found that actin-activated ATPase activity of V1 isolated from left ventricle of rat or rabbit was 2-3 times higher than that of V3 [8]. *In vitro* motility experiments showed that isomyosin V1 moved the actin filament 2-3 times faster than isoform V3 [9, 10]. Differences in an average isometric force of these isomyosins were dependent upon animal type, i.e. force generated by these isoforms was the same (rat) [11], or V3 was two times more powerful (rabbit) [9].

The interaction of single myosin and actin molecules studied by means of a dual laser trap setup indicated that no differences in either unitary actin displacement or force were observed between isoforms. The duration of unitary displacement events was longer for the V3 isoform [12, 13].

Differences in isoform ratio V1/V3 in cardiomyocyte are directly manifested in different velocity characteristics of tension development: upon domination of rapid isoform V1, tension develops faster in cardiac muscle cell. Besides the direct contribution to myocardium mechanical function (as a power-generating units), different isomyosin ratios are also important as they affect

Abbreviations: CaTnC) calcium–troponin C complex; TnC) troponin C; OQS) oxygen-quenching system.

* To whom correspondence should be addressed.

cooperatively troponin C (TnC) affinity to calcium. It is known that calcium entering cytosol binds a regulatory protein—TnC. As a result tropomyosin then shifts from exposed binding sites on actin filaments, so myosin heads might attach, i.e. cross-bridges form. More calcium enters the cell, more calcium–troponin C complexes (CaTnC) form and more power-generating cross-bridges appear. In turn, cross-bridges affect CaTnC binding/dissociation kinetics constants: the more cross-bridges formed along an acting filament, the higher affinity of TnC for calcium [14]. This cooperative interaction of regulatory and contractile proteins is a key mechanism explaining a number of biomechanical phenomena of active myocardium, connected with the influence of mechanical conditions of contractions on the activation of cardiac muscle [15].

Cooperativity mechanisms in cross-striated muscles have traditionally been studied on skinned samples. Experiments are usually performed to study the effects of different calcium concentrations on velocity and force: pCa–velocity and pCa–force relationships [14]. Recently *in vitro* motility assay with reconstructed thin filaments (consisting of actin, troponin, and tropomyosin proteins) was also used to study the regulation of power generation processes in muscles by calcium. In particular, this method provided an analog of the pCa–force relationship *in vitro* [16]. This approach was used in the present study to investigate the contribution of the cooperativity of contractile and regulatory proteins to the regulation of myocardium contractions.

MATERIALS AND METHODS

Isolation of isomyosins V1 and V3 from left cardiac ventricles of hyper- and hypothyroid rabbits. Myocardium of left ventricles of hyperthyroid and hypothyroid rabbits contains predominantly myosin V1 and V3 isoforms, respectively. To obtain pure isoforms of myocardial myosins, we treated experimental animals as follows. Hyperthyroidism was induced by intramuscular injections of L-thyroxine (Sigma, USA) (0.2 mg/kg) in 2-month-old rabbits (weight 1.5 kg) during two weeks. Animals were rendered hypothyroid with propylthiouracyl-supplemented drinking water (0.8 mg/ml) for three weeks [9]. At the end of the medication period, left and right cardiac ventricles were isolated and rapidly frozen separately in liquid nitrogen. Samples were kept at –86°C.

Myosin from rabbit heart was obtained according to a standard procedure [17] with minor modifications. For storage, myosin solution was mixed with cold glycerol in 1 : 1 (v/v) ratio and kept at –20°C. Myosin/glycerol mixture was stirred with deionized water in 1 : 10 ratio on the day of the experiment. In 1 h myosin was pelleted by centrifugation at 10,000*g* for 20 min and dissolved with an equivalent volume (w/v) of high ionic strength buffer. Nonfunctional myosin molecules were separated using the following approach. Myosin solution was added to actin filaments (F-actin) at molar ratio of 1 : 1.5 and ATP to 1 mM. The mixture was centrifuged at 600,000*g* for 20 min. During this process, myosin molecules containing damaged heads were irreversibly bound to F-actin and precipitated, while the supernatant solution contained only functional myosin molecules. The resulting myosin preparation was used during the same experimental day.

Determination of content of α- and β-heavy chains in cardiac myosin using SDS-PAGE. The detailed protocol of SDS-PAGE was kindly provided by Dr. J. van der Velden (VU University Medical Center, Amsterdam, The Netherlands) and used with minor modifications [18]. After electrophoresis, gels were stained with silver and washed with water. Gels were scanned with a Bio-Rad (USA) densitometer, and percent ratio of α- and β-heavy myosin chains in the sample was determined.

Preparation of actin, troponin, and tropomyosin. Actin, troponin, and tropomyosin were isolated from an acetone powder obtained according to a standard procedure [19] from psoas muscles of rabbit (in case of actin) and from left ventricular myocardium of bovine heart (in case of troponin and tropomyosin). Actin was isolated from the acetone powder (skeletal) according to a standard protocol [19]. The actin was dyed with rhodamine phalloidine (Sigma). Troponin and tropomyosin were isolated from the acetone powder from bovine heart according to the protocols suggested by Potter [20] and Smillie [21] with minor modifications. The purity of the resulting protein preparations was tested by SDS-PAGE. After dialysis, troponin and tropomyosin were frozen in aliquots in liquid nitrogen and kept at –86°C. A troponin aliquot was used for two months and tropomyosin for four months. Proteins were isolated at 4°C.

Preparation of reconstructed thin filaments. Thin filaments were reconstructed from actin, troponin, and tropomyosin by mixing these proteins in the following concentrations: 400 nM rhodamine phalloidine dyed F-actin, 80 nM troponin, and 100 nM tropomyosin at 4°C in buffer AB: 25 mM KCl, 25 mM imidazole, 4 mM $MgCl_2$, 1 mM EGTA, and 10 mM DTT, pH 7.4 [22-24]. Filaments were incubated for 1 h before use and were used in experiments for 1 week. Protein ratios in thin filaments were tested by SDS-PAGE.

***In vitro* motility assay with reconstructed thin filaments.** *In vitro* motility assay was used to measure the velocity of actin/thin filament movement on a surface covered with a motor protein; motor protein force was estimated with a glass micro needle [9] or with actin-binding proteins [25, 26].

The procedure of velocity assessment for unloaded movement of thin filaments was described in detail in our previous work on measurements of thin filament movement velocity at different calcium concentrations in solu-

tion [27]. In the present study, we focus on evaluation of force for heart myosin isoforms.

Plotting pCa–force relationship. Force generated by myosin cross-bridges attached to actin filaments was determined using actin-binding protein, α-actinin, as an internal load. This method explores the fact that α-actinin immobilized on the surface of a flow cell can bind thin filaments and stop their movement. The higher the force generated by myosin heads, the more α-actinin is needed to stop the thin filament. To determine isometric force of myosin bridges, α-actinin quantity on the surface of the flow cell was sequentially increased until the total shut-down of all thin filaments. Isometric force was proportional to the minimal concentration of α-actinin loaded in the cell needed for the total shut-down of the thin filaments.

In a series of experiments pCa–force relationship was obtained at different calcium concentrations, and the ratio of stopping quantity of α-actinin at a given calcium concentration to that at saturating calcium concentration was used as the force characteristic. The data are fitted well with a sigmoid curve (Fig. 1). This result corresponds to the shape of pCa–force relationship obtained in numerous experiments on skinned skeletal and cardiac muscles [14]. It is known that cooperativity appears as a slope of pCa–force relationship in the central linear part of the sigmoid, and the tangent of the angle represents the Hill cooperativity coefficient. The Hill equation corresponding to a sigmoid curve in semilogarithmic coordinates can be presented as:

$$F = 1/[1 + 10^{(-h(\mathrm{pCa}_{50} - \mathrm{pCa}))}],$$

where F is a dimensionless force expressed as parts of F_{max}, h is the Hill coefficient, and pCa_{50} is pCa value at which half-maximal force is achieved.

The experimental protocol was as follows. A 50-μl sample of heart myosin at concentration(s) 200(300) μg/ml was loaded in the flow cell; protein was attached to the surface covered with nitrocellulose (Sigma).

The flow cell was then washed with high (AB with 0.5 M KCl) and low ionic strength buffer AB, and then α-actinin in AB buffer was loaded at given concentration and incubated for 10 min. Then 50 μl of 0.5 mg/ml BSA were added and the mixture incubated for 1 min. Further, 500 μg/ml of non-dyed F-actin in AB+ (AB with 2 mM ATP) was added and incubated for 5 min to block nonfunctional myosin heads. The flow cell was washed three times with AB buffer. Then 50 μl of 10 nM rhodamine phalloidine dyed thin filament in AB buffer containing 100 nM troponin and 100 nM tropomyosin were added with subsequent incubation for 10 min.

The cell was washed with AB buffer containing BSA (0.5 mg/ml) and "oxygen-quenching system" (OQS): glucose (3.5 mg/ml), catalase (0.02 mg/ml), glucose oxidase (0.15 mg/ml), and DTT (20 mM). After that, it was possible to observe a rigor connection of thin filament with myosin. Final solution containing AB+/BSA/OQS, 100 nM troponin, 100 nM tropomyosin, and free calcium at different concentrations was added. If all the components were working, thin filament movement on myosin was observed.

Experiments were performed at concentrations of free calcium (pCa) from 4 to 8 in the flow cell. The necessary concentration of free calcium was achieved by the addition of the corresponding quantity of $CaCl_2$, calculated with the free on-line software WEBMAXC STANDARD (http://www.stanford.edu/~cpatton/webmaxc/webmaxcS.htm), into the final solution.

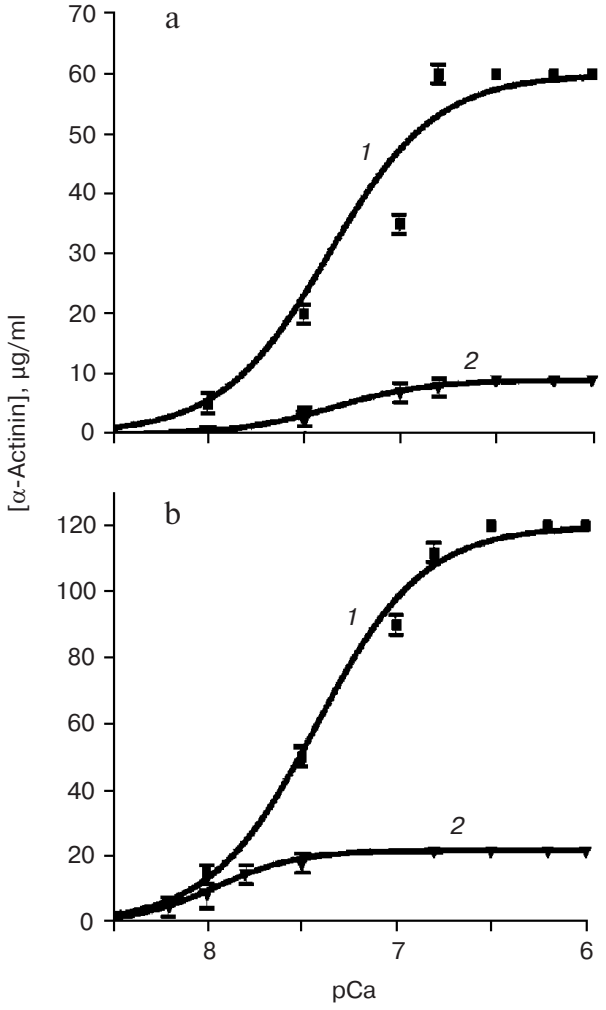

Fig. 1. Representative graphs of the effect of calcium on α-actinin concentration in semilogarithmic coordinates. a) Curves for V1 at myosin concentrations of 300 (*1*) and 200 (*2*) μg/ml; b) the same curves for isomyosin V3. α-Actinin concentration obtained in *in vitro* motility assays at different isomyosins concentrations: triangles (200 μg/ml), squares (300 μg/ml). α-Actinin concentration represents the mean value ± SD from three experiments. Experimental data was fitted with the Hill equation (see "Materials and Methods").

All experiments were performed at 27°C.

Data analysis was performed using GMview software developed in the National Institute of Medical Research (G. Mashanov, MRC NIMR, London, England).

RESULTS

In vitro motility assay experiments with reconstructed thin filament were performed to obtain pCa–α-actinin relationship for different concentrations of isomyosins V1 and V3. Representative graphs for these relationships at isomyosins concentrations in the flow cell equal to 200 and 300 μg/ml are shown in Fig. 1. At saturating concentration of calcium, the α-actinin quantity in solution that totally stopped the movement of all thin filaments was 6 times higher for 300 μg/ml as compared to that for 200 μg/ml for both types of myosin. It is noteworthy that the α-actinin quantity that totally stopped the movement of all filaments was exactly 2 times higher for isomyosin V3 compared to V1 for both concentrations used.

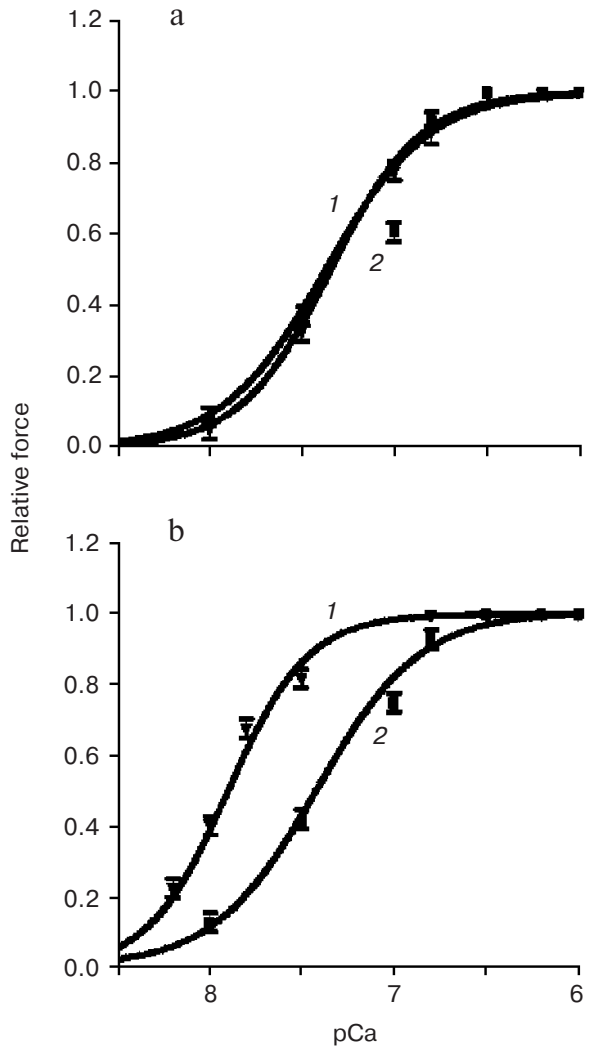

Fig. 2. Representative graphs of the effect of free calcium on the relative force in semilogarithmic coordinates. a) Curves for V1 at myosin concentrations of 200 (*1*) and 300 (*2*) μg/ml; b) same curves for isomyosin V3. Relative force determined by *in vitro* motility assays at different isomyosins concentrations: triangles (200 μg/ml), squares (300 μg/ml). Relative force is presented as a mean value ± SD from three experiments.

Figure 2 shows representative graphs for pCa–force relationships obtained by normalization of α-actinin concentrations from Fig. 1 on the corresponding maximal concentration of this protein (see "Materials and Methods"). Hill coefficients for pCa–force curves presented in panel (a) for isomyosin V1 concentrations of 200 and 300 μg/ml were 1.76 and 1.56, respectively. The Hill coefficients were 2.00 and 1.54 for isomyosin V3 (panel (b)) concentrations of 200 and 300 μg/ml, respectively. Therefore, cooperativity increases with a decrease in the protein concentration.

The same data regrouped to compare pCa–force curves for the same concentration of different isomyosins in the flow cell are presented in Fig. 3. Hill coefficients for pCa–force (panel (a)) relationship at 300 μg/ml were 1.56 and 1.54 for isomyosins V1 and V3, respectively. At 200 μg/ml (panel (b)), Hill coefficients were 1.76 for V1 and 2.00 for V3. Thus, the Hill coefficient was the same for both isoforms at 300 μg/ml. However, it was higher for V3 compared to V1 at lower concentration (200 μg/ml).

Calcium sensitivity estimated as pCa_{50} (pCa producing half-maximal force) did not significantly differ for the isoforms at 300 μg/ml—7.32 and 7.42 for V1 and V3, respectively. However, at 200 μg/ml the difference in calcium sensitivity was somewhat higher—7.34 for V1 and 7.93 for V3.

DISCUSSION

In vitro motility assay with regulated thin filament allows reconstructing pCa–force relationship on the level of interacting proteins. It allows elimination of artifacts caused by passive mechanical properties of the muscle and cardiomyocyte and study of the interaction of thin filament with myosin *per se*. Also, this method allows investigation of both pure isomyosin isoforms (V1 and V3) separately and their mixtures. The concentration of actin binding protein (α-actinin) stopping thin filament movement was used in these experiments as a force indicator.

It was shown that increasing concentration of both V1 and V3 from 200 to 300 μg/ml at saturating concentration of calcium increased α-actinin stopping quantity six-fold. Therefore, force is not changing proportionally with increase of protein concentration, but is rising more sharply.

It was found in our experiments that α-actinin stopping quantity at saturating concentration of calcium was exactly 2 times higher for V3 than for V1. Therefore, these

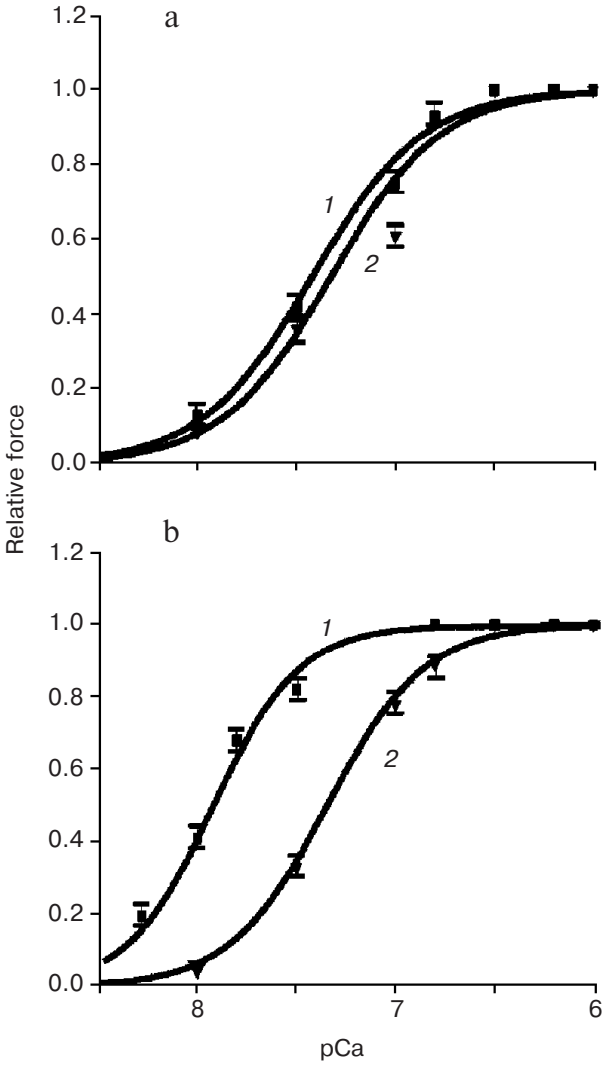

Fig. 3. Representative graphs of the effect of free calcium on the relative force for V1 (*2*) and V3 (*1*) at the same myosin concentrations on the flow cell surface in semilogarithmic coordinates. Myosin concentration was 300 (a) and 200 μg/ml (b). Relative force values are shown as triangles for isomyosin V1 and as squares for isomyosin V3.

results are in good agreement with the experimental data obtained by other investigators by *in vitro* motility assays both with unregulated filament [9, 11] and with reconstructed thin filament [16]. For the unregulated thin filament, the same result was obtained at different myosin concentrations. However, earlier published data on regulated thin filament stopped with α-actinin [16] were obtained with only one concentration of isomyosins. Here we obtained the corresponding results for two isomyosin concentrations—200 and 300 μg/ml.

It is known that cooperativity mechanisms in contractile and regulatory proteins are important in calcium regulation of myocardial contractions. There are different types of cooperativity. It was shown that two of them play a key role in the regulation of contractions.

1. Cross-bridge–troponin cooperativity (Xb–TnC): strong binding of cross-bridges (myosin heads interacting with actin) with thin filament at any regulatory group consisting of seven actin monomers, tropomyosin, and troponin increases troponin C affinity to Ca^{2+} in this and neighboring regulatory groups.

2. Troponin–troponin cooperativity (TnC–TnC): Ca^{2+} binding with one TnC increases calcium binding with neighboring TnC [14].

As shown in a mathematical model of regulation of myocardial contractions [15, 28], cooperativity provides a basis for all known effects of mechanical conditions on calcium activation of myocardial contractions. It is also involved in mechano-electric feedback in cardiomyocytes [29]. Thus, the key role of both types of cooperativity in effects of load-dependent relaxation and fast filament deformations during isometric contractions was demonstrated. As shown in the model, Xb–TnC cooperativity is the main mechanism responsible for these effects, while TnC–TnC cooperativity substantially enhances them.

Obtaining of pCa–force relationship is one of the main experimental approaches of studying mechanisms of cooperativity in muscles. The slope of the central part of this relationship, Hill coefficient, is an integral characteristic of all cooperativity types inherent of cardiomyocyte. The pCa–force relationship is traditionally registered on skinned filament or cardiomyocyte [14].

Comparison of Hill coefficient for V1 and V3 is important since different myosin isoforms are present in heart, and cardiomyocytes containing predominantly V1 or V3 is found in different heart regions.

Using *in vitro* motility assay with reconstructed thin filament, we obtained pCa–force relationships for different concentrations of isomyosins V1 and V3 and analyzed the corresponding Hill coefficients (*h*). It was found that *h* values do not differ significantly for these two isoforms at protein concentration of 300 μg/ml (1.56 and 1.54 for V1 and V3, respectively), and the Hill coefficient is higher for V3 at isoform concentration of 200 μg/ml (2.00 for V3 and 1.76 for V1). Therefore, differences in Hill coefficients for different isomyosins were obtained at lower protein concentration. It was also found that Hill coefficient increases when protein concentration decreases for both isoenzymes. These data were obtained for the first time. In a previously published paper [16], the ratio pCa–force was estimated for isomyosins V1 and V3, but the data were presented for only one myosin concentration. In the published protocol, the authors did not show what this concentration was. This indicates that myosin concentration was not considered as a factor affecting the slope of the relationship under consideration and calcium sensitivity.

To understand the revealed influence of isomyosin concentration on characteristics of pCa–force relationship, it is necessary to consider the fact that lowering myosin concentration affects pCa–force dependence slope in two opposite ways.

1. Saturating concentration of calcium at given myosin quantity on the surface of the flow cell needed to provide the maximal force is decreasing. This factor causes the sigmoid to turn counter-clockwise.

2. Cooperative influence of cross-bridges on Ca affinity for TnC and finally actin affinity for myosin is decreasing. This factor turns the sigmoid clockwise.

The total effect of myosin concentration decrease represented as counter-clockwise turn means that first way is predominant. The total effect turning the curve clockwise represents a second tendency. The unaffected slope of pCa–force relationship at decreasing myosin concentration indicates that the two tendencies equilibrate each other.

Taking this consideration into account, we compared pCa–force sigmoid for V1 and V3 at 200 and 300 μg/ml. For both curves, counter-clockwise turn was observed when decreasing myosin concentration. Therefore, the first tendency is predominant for both V1 and V3, but its extent is higher for V3. This fact suggests that cooperative dependence of CaTnC kinetics upon V1 is stronger than upon V3.

Obviously, the slope of the pCa–[CaTnC] relationship obtained together with pCa–force relationship under the same experimental conditions might be a direct index of the contribution of isomyosins V1 or V3 to the kinetics of calcium–troponin complexes (through Xb–TnC cooperativity). Apparently, pCa–force slope cannot be unambiguously used to estimate the slope of the pCa–[CaTnC] relationship. Our data on differences in pCa–force slopes at different myosin concentrations confirm this conclusion as well as the following considerations. The pCa–force relationship under stationary conditions coincides with the pCa–[N] relationship with normalization accuracy (N is the concentration of myosin cross-bridges). Simple mathematical calculations show that the relationship between [CaTnC] and corresponding bridges concentration is nonlinear and depends upon such two parameters as affinity constant of actomyosin complex and myosin concentration. That is:

$$[\mathrm{CaTnC}] = \frac{(N/\overline{N})}{k_a\,(1 - (N/\overline{N}))},$$

where k_a is affinity constant and $\overline{N}$ is myosin concentration in the system (i.e. in cardiomyocyte or *in vitro* motility assay).

Both of the two parameters could be different for different isomyosins. There is still a possibility that the greater slope of pCa–force (for V1 isoform compare to V3 isoform) is transformed into smaller slope of pCa–[CaTnC] relationships (when comparing the same isoforms in the same experiments).

The given analysis indicates that slope of pCa–force relationship does not give an unambiguous answer to the question about the influence of cross-bridges on CaTnC kinetics, and, therefore, does not allow making a conclusion about different isomyosin contributions to calcium regulation of myocardial contractions.

Thus, a question about differences in cooperative influence of cross-bridges formed by different isoforms of cardiac myosin on CaTnC kinetics could be correctly answered only by direct registration of pCa–[CaTnC] relationship under the same experimental conditions as pCa–force. For example, by further development of the given method it could be possible to measure up- and down-stream concentrations of calcium (before and after the flow cell). The simultaneous registration of pCa–force and pCa–[CaTnC] relationships substantially differ from the known experiments in solution [30] in the following.

1. It provides an understanding of any correlation (and its nature) between Hill coefficients for these two relationships.

2. It makes possible study of cooperative protein interactions in systems performing mechanical work, i.e. under conditions closer to that in cardiomyocytes compared to thin filaments placed in physiological solution.

Authors would like to thank Dr. G. I. Mashanov (MRC NIMR, London, England) for the provided software and Dr. J. van der Velden (VU University Medical Center, Amsterdam, The Netherlands) for the detailed protocol of SDS-PAGE.

This study was supported by the Russian Foundation for Basic Research (grant 05-04-48353) and by a grant of the President of the Russian Federation No. 4923.2006.4 for Supporting Leading Scientific Schools (NSh 4923.2006.4).

REFERENCES

1. Hoh, J. F. Y., McGrath, P. A., and Hale, P. (1977) *J. Mol. Cell Cardiol.*, **10**, 1053-1076.
2. Sartore, S., Gorza, L., Pierobon Bormioli, S., Dalla Libera, L., and Schiaffino, S. (1981) *J. Cell Biol.*, **88**, 226-233.
3. Litten, R. Z., Martin, B. J., Buchthal, R. H., Nagai, R., Low, R. B., and Alpert, N. R. (1985) *Circ. Res.*, **57**, 406-414.
4. Samuel, J. L., Rappoport, L., Mercadier, J. J., Lompre, A. M., Sartore, S., Triban, C., Schiaffino, S., and Schwartz, K. (1983) *Circ. Res.*, **52**, 200-209.
5. Alpert, N. R., Mulieri, L. A., Hasenfuss, G., and Holubarsch, Ch. (1994) in *Myocardial Optimization and Efficiency, Evolutionary Aspects and Philosophy of Science Consideration* (Burkhoff, D., ed.) Springer, Steinkopff-Darmstadt-N. Y., pp. 30-40.
6. Lompre, A. M., Schwaetz, K., d'Albis, A., Lacombe, G., van Thiem, N., and Swynghedauw, B. (1979) *Nature*, **282**, 105-107.
7. Van der Velden, J., Borgdorff, P., and Stienen, C. J. M. (1999) *Cell Mol. Life Sci.*, **55**, 788-798.

8. Pope, B., Hoh, J. F. Y., and Weeds, A. (1980) *FEBS Lett.*, **118**, 205-208.
9. Van Buren, P., Harris, D. E., Norman, R. A., and Warshaw, D. M. (1995) *Circ. Res.*, **77**, 439-444.
10. Sata, M., Sugiura, S., Yamashita, H., Momomura, S., and Serizawa, T. (1993) *Circ. Res.*, **73**, 696-704.
11. Malmqvist, Ulf. P., Aronsham, A., and Lowey, S. (2004) *Biochemistry*, **43**, 15058-15065.
12. Sugiura, S., Kobayakawa, N., Fujita, H., Yamashita, H., Momomura, S., Chaen, S., Omata, M., and Sugi, H. (1998) *Circ. Res.*, **82**, 1029-1034.
13. Palmiter, K. A., Tyska, M. J., Dupius, D. E., Alpert, N. R., and Warshaw, D. M. (1999) *J. Physiol.*, **519**, 669-678.
14. Gordon, A. M., Homsher, E., and Regnier, M. (2000) *Physiol. Rev.*, **80**, 853-924.
15. Izakov, V. Ya., Katsnelson, L. B., Blyakhman, F. A., Markhasin, V. S., and Shklyar, T. F. (1991) *Circ. Res.*, **69**, 1171-1184.
16. Noguchi, T., Camp, P. Jr., Alix, S. L., Gorga, J. A., Begin, K. J., Leavitt, B. J., Ittleman, F. P., Alpert, N. R., LeWinter, M. M., and van-Buren, P. (2003) *J. Mol. Cell Cardiol.*, **35**, 91-97.
17. Margossian, S. S., and Lowey, S. (1982) *Meth. Enzymol.*, **85**, 55-71.
18. Narolska, N. A., Eiras, S., van Loon, R. B., Boontje, N. M., Zaremba, R. S., Berg, S. R., Stooker, W., Huybregts, M. A., Visser, F. C., van der Velden, J., and Stienen, G. J. (2005) *J. Muscle Res. Cell Motil.*, **26**, 39-48.
19. Pardee, J. D., and Spudich, J. A. (1982) *Meth. Enzymol.*, **85**, 164-181.
20. Potter, J. D. (1982) *Meth. Enzymol.*, **85**, 241-263.
21. Smillie, L. B. (1982) *Meth. Enzymol.*, **85**, 234-241.
22. Homsher, E., Kim, B., Bobkova, A., and Tobacman, L. S. (1996) *J. Biophys.*, **70**, 1881-1892.
23. Gordon, A. M., LaMadrid, M. A., Chen, Y., Luo, Z., and Chase, P. B. (1997) *J. Biophys.*, **72**, 1295-1307.
24. Fraser, I. D. C., and Marston, S. B. (1995) *J. Biol. Chem.*, **270**, 7836-7841.
25. Janson, L. W., Sellers, J. R., and Taylor, D. L. (1992) *Cell Motil. Cytoskel.*, **22**, 274-280.
26. Bing, W., Knott, A., and Marston, S. (2000) *Biochem. J.*, **350**, 693-699.
27. Kopylova, G. V., Katsnelson, L. B., Ovsyannikov, D. A., Bershitskiyi, S. Yu., and Nikitina, L. V. (2006) *Biophysics*, **51**, 687-691.
28. Katsnelson, L. B., and Markhasin, V. S. (1996) *J. Mol. Cell Cardiol.*, **28**, 475-486.
29. Markhasin, V. S., Solovyova, O. E., Katsnelson, L. B., Protsenko, Y. U., Kohl, P., and Noble, D. (2003) *Prog. Biophys. Mol. Biol. Focussed Issue on MEF*, **82**, 207-220.
30. Grabarek, Z., Grabarek, J., Leavis, P. C., and Gergely, J. (1983) *J. Biol. Chem.*, **258**, 14098-14102.