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Functional effects of substitutions I92T and V95A in actin-binding period 3 of tropomyosin

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ABSTRACT

Tropomyosin polymerizes along actin filaments and together with troponin regulates muscle contraction in a Ca-dependent manner. Actin-binding periods are homologous residues, which repeat along tropomyosin sequence, form tropomyosin-actin interface and determine regulatory functions. To learn how period 3 is involved in tropomyosin functions we examined effects of two mutations in Tpm1.1, I92T and V95A, which have been linked to dilated and hypertrophic cardiomyopathies characterized respectively by hyper- and hypocontractile phenotypes. In this work the functional consequences of both mutations were studied *in vitro* by using actin thin filaments reconstituted in the presence of mutant Tpm1.1 homodimers carrying the substitutions in both tropomyosin chains, Tpm1.1 heterodimers with substitution only in one Tpm1.1 chain, and Tpm1.1/Tpm2.2 heterodimers with substitution in Tpm1.1 chain and wild type Tpm2.2 in the second chain. The presence of the substitution I92T decreased the tropomyosin affinity for actin, abolished Ca^{2+}-dependent activation of the actomyosin ATPase, decreased the sensitivity of the tropomyosin-troponin complex to subsaturating Ca^{2+} concentrations and reduced the cooperativity of the myosin-induced transition of the thin filament to a fully active state. The substitution V95A had opposite effects: increased actin affinity, increased the actomyosin ATPase activity above the level observed for wild type Tpm and increased cooperativity of myosin-induced activation of the thin filaments reconstructed with homo- and heterodimers of tropomyosin. Substitutions I92T and V95A were dominant, but the formation of heterodimers modified the effects observed for homodimers.

1. Introduction

Contraction is based on ATP hydrolysis driven interactions between myosin heads and actin, which result in sliding the myosin thick filaments past thin filaments. In striated muscle the process is tightly controlled in a Ca-dependent manner by tropomyosin (Tpm) and troponin complex (Tn), the thin filament regulatory proteins. Tropomyosin polymerizes end-to-end on both sides of actin filament to form a continuous cable that anchors troponin complex. Upon stimulation Ca^{2+} is released from the sarcoplasmic reticulum and binds to TnC, the Ca-binding subunit of Tn. This triggers conformational changes within the thin filament, including an azimuthal shift of tropomyosin chains on actin from blocked (B) to closed (C) state, which facilitates interactions between actin and myosin heads. Isomerization of myosin heads from weak to strong actin binding state causes a further shift of tropomyosin chains into the myosin-induced open state (M), which results in full contraction. The removal of Ca^{2+} from sarcoplasm reverses the sequence of reactions leading to relaxation [1–5].

Tropomyosin is a two-chain coiled coil, which is characterized by repeats of seven amino acid residues (*a-b-c-d-e-f-g*) with hydrophobic residues located at the *a* and *d* position in each α-helical chain. Interactions between hydrophobic residues stabilize the core of the coiled coil. Tropomyosin contacts with seven consecutive actin monomers are stabilized by repeats of homologous residues exposed outside the coiled coil in positions *b*, *c* and *f* of the heptapeptide, which form seven actin-binding periods [6,7]. Specific contacts at the tropomyosin-actin interface are important determinants of the three states of the thin filament [8].

In human striated muscle three main tropomyosin isoforms, Tpm1.1, Tpm2.2 and Tpm3.12 are present. The relative amounts of the

Abbreviations: DCM, dilated cardiomyopathy; HCM, hypertrophic cardiomyopathy; Tpm1.1, homodimer of Tpm1.1 isoform; Tpm2.2, homodimer of Tpm2.2; His-Tpm2.2, homodimer of His-tagged Tm2.2; Tpm1.1/Tpm2.2, heterodimer of two tropomyosin isoforms; Tpm1.1-I92T/Tpm1.1, Tpm1.1-I92T/Tpm2.2, Tpm1.1-V95A/Tpm1.1, Tpm1.1-V95A/Tpm2.2

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isoforms vary depending on the type of muscle [9]. The isoforms form homo- and heterodimers in different proportions [10,11]. In human heart the predominant tropomyosin isoform is Tpm1.1. According to different studies, the amount of Tpm2.2 varies between 3 and 8% of total tropomyosin expressed in different parts of myocardium [12–14]. The affinities of homodimers for actin and their ability to regulate actin-myosin interactions can be distinct from heterodimers [10], which increases the functional diversity among tropomyosins expressed in different tissues.

At first glance tropomyosin appears as a steric blocker, which is passively shifted from a position blocking the access of myosin heads to an activating position facilitating myosin binding. This is in fact oversimplified, because tropomyosin actively participates in all activation steps. One parameter, which determines the equilibrium between different positions of tropomyosin on actin is the affinity of tropomyosin to actin in each of the activation states, which are stabilized by specific tropomyosin-actin interactions (reviewed in [8]). The second parameter, which determines the position tropomyosin assumes on the filament, is the cooperativity of the transition between activation states. Cooperativity is a complex process, which depends on the strength of the end-to-end interactions between neighboring tropomyosin molecules and flexibility of inner regions of tropomyosin [15]. The actin-binding period 3 of tropomyosin was shown to be critical for tropomyosin binding in different activation states and Ca-dependent activation of the actomyosin ATPase [16–19]. However, a detailed description of the structural determinants of cooperativity is still missing.

In order to gain insight into the complexity of the tropomyosin-dependent regulatory mechanisms, in this work we examined the effects of two mutations in TPM1, the gene encoding Tpm1.1, which were associated with human cardiomyopathy. Mutations Ile92Thr (I92T) and Val95Ala (V95A) are located next to each other in actin-binding period 3 at the interface between two tropomyosin chains (Fig. 1) and substitute bulky hydrophobic residues for either hydrophilic Thr or hydrophobic, but small Ala (Fig. 1B). In spite of this close and equivalent localization, the substitutions cause different phenotypes – I92T was found in patients with dilated cardiomyopathy (DCM), whilst V95A was associated with hypertrophic cardiomyopathy (HCM) [20,21]. One of the major trait of DCM is hypocontraction, which is in contrast to hypercontraction characteristic for HCM [22]. This raises a question: which tropomyosin functions are affected by the substitutions that result in the hypo- or hypercontractile phenotype? The effects of V95A were analyzed previously in several studies, which showed destabilization of the coiled coil [23], reduced flexibility and actin affinity [24], increased sensitivity to subactivating Ca^{2+} concentrations [21,23] and impaired relaxation of the myocardium [25]. The effects of I92T on tropomyosin functions are still not known. Because both mutations are heterozygous [22], in cardiomyocytes mutant Tpm1.1 are expressed along with the wild type Tpm1.1 and Tpm2.2.

In this work the effects of substitutions I92T and V95A on Tpm1.1 functions were compared in several *in vitro* assays. Biochemical analyses showed that the substitutions affected the regulation of actin-myosin interactions in opposite ways – while I92T reduced the sensitivity of the thin filament to activating Ca^{2+} concentration and inhibited interactions between actin and myosin, V95A sensitized the regulatory proteins to Ca^{2+} and potentiated the actomyosin interactions. The effects of the substitutions were not attenuated by the presence of the wild type tropomyosin chains in the heterodimers. The results show that actin-binding period 3 is genuinely involved in the regulation of actomyosin interactions. However functional consequences of the mutations cannot be easily predicted based on the positions of the substitutions in tropomyosin coiled coil. *In vitro* studies provide valuable data, which give insight into the molecular mechanism of contraction regulation.

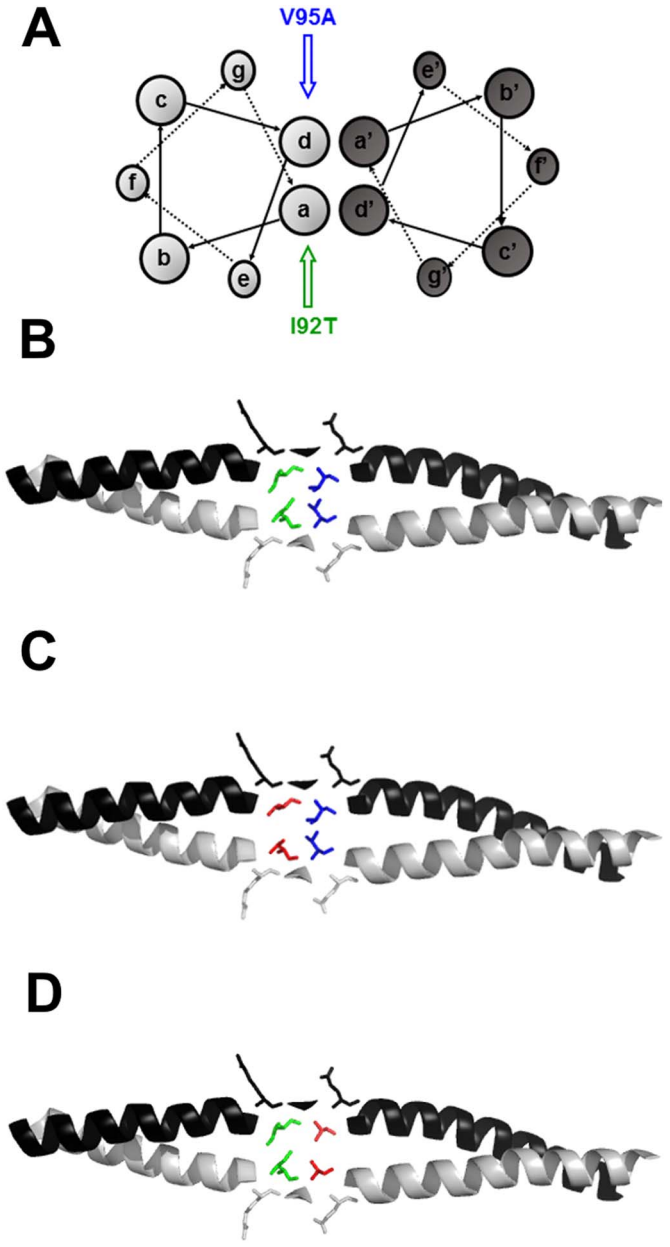

Fig. 1. Localization of I92T and V95A substitutions in the coiled coil heptapeptide repeat and in the coiled coil structure of Tpm1.1 segment comprising residues 68–125. (A) The diagram shows two helical turns of seven amino acids (marked from a to g) in two-chain coiled coil. Each first (a and a′) and fourth (d and d′) residue is part of a hydrophobic core of the coiled coil. Each of the mutations can be present either in one or two chains. (B) Site view with stick marked (black and grey) consensus actin-binding site residues R90 and E97 and core residues I92 (green) and V95 (blue). (C) Site view showing the I92T substitution (red). (D) Site view showing the V95A substitution (red). The wild type fragment sequence of Tpm1.1 was from the Protein Data Bank, accession number 2TMA, visualized in PyMol. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2. Materials and methods

2.1. Construction of plasmids carrying cDNA encoding human Tpm1.1 and Tpm2.2 and Tpm1.1 cardiomyopathy mutants

To obtain cDNA, which translated into amino acid sequence identical with human cardiac tropomyosin Tpm1.1 (NCBI ref seq: NP_689476.2), Lys220 in rat Tpm1.1 cDNA was substituted for Arg (K220R). Rat Tpm1.1 template cDNA contained Met-Ala-Ser codons at the 5′ end to produce a protein with the N-terminal AlaSer extension,