Abstract

Skeletal muscle is carefully regulated unit composed of smaller components acting in cooperation to perform its primary function - movement. It is comprised of repetitive isotropic and anisotropic bands, giving it a striped appearance. The smallest periodic unit, termed sarcomere, is composed of actin and myosin filaments, which together with other proteins form sarcomeric cytoskeleton. On both ends, sarcomere is delimited with the Z-discs, which act as transducers of the generated tension.

Some of the sarcomere components are actin binding proteins from the myotilin/myopalladin/palladin family. In this thesis, we have focused on the myotilin and from the structural perspective shed light on its free and bound form in interaction with actin. Ig domains, presenting the only structured part of the molecule, are responsible for the binding to actin. With the use of small-angle X-ray scattering (SAXS) and molecular dynamics simulations we have shown that Ig domains preferentially adopt semi-extended orientation in the free form, however with a degree of flexibility. This plays an important role in the binding partner recognition, as a part of the *induced fit* mechanism.

We have confirmed the interaction and identified interaction sites on myotilin and actin *in vitro*, using the complementary methods of structural biology and biochemistry. Moreover, we have determined disociation constants for binding of different myotilin constructs to actin, where binding to F-actin showed significantly stronger affinity (9.2 ± 1.0 μM) compared to the interaction with G-actin (89 ± 10 μM). Myotilin mutants showed up to 70 % decreased binding to the F-actin, which further confirmed the previously identified binding site. Based on the experimental data, we prepared model of myotilin : F-actin complex using molecular docking. In the model, Ig domains are binding to the subdomain 1 of each actin protomer, leading to the stabilization of both binding partners.

In the subsequent experiments we also showed that myotilin Ig domains are able to influence actin dynamics by increasing its polymerization rate and decreasing the rate of depolymerization. Finally, using C2C12 and A7r5 cell lines, we unequivocally confirmed the interaction and binding site *in vivo*, extending previous findings with the FRAP experiments that showed increased dynamics of the actin-binding mutants, compared to the wild-type.

In conclusion, the gathered results offer a first insight into the myotilin interaction properties on the structural level, with the first detailed depiction of Ig domain binding to the actin filament. Furthermore, they serve as an excellent foundation for the future experiments regarding the role of myotilin in tissue remodelling in the light of the understanding the mechanisms of the sarcomere action.

Key words: striated muscle, Z-disc, myotilin, actin, Ig domains, SAXS, FRAP.