Abstract

The activity of several cytosolic proteins critically depends on the concentration of calcium ions. One of the most important intracellular calcium-sensing proteins is α-actinin-1, the major actin crosslinking protein in focal adhesions and stress fibers. The actin crosslinking activity of α-actinin-1 has been proposed to be negatively regulated by calcium, but the underlying molecular mechanisms are poorly understood. The object of our research was α-actinin-1 and its structural and calcium-binding properties.

For this reason we prepared full-length α-actinin-1, half dimer and calmodulin-like domain (CaMD) which was also prepared as mutant forms with mutations in putative calcium-binding sites using *E. coli* expression system. Mutagenesis experiments, coupled with isothermal calorimetry and mass-spectrometry data designed to validate the calcium binding stoichiometry and binding site, showed that human non-muscle α-actinin-1 binds a single calcium ion in EF1 within the N-terminal lobe. We have determined the dissociation constant for calcium binding by CaMD with its value of 104.2 ± 15.4 μM.

In association with the group of Prof. Dr. Janez Plavec from Slovenian NMR Centre in Ljubljana we determined the first high-resolution NMR structure of calmodulin-like domain in calcium-bound and calcium-free form. These structures reveal that in the absence of calcium, CaMD displays a conformationally flexible ensemble that undergoes a structural change upon calcium binding, leading to limited rotation of the N- and C-terminal lobes around the connecting linker and consequent stabilization of the calcium-loaded structure.

We have also prepared crystals of half dimer α-actinin-1 in the absence of calcium and recorded a set of diffraction data with the highest resolution of 3.63 Å. However, building of a complete structure was not possible due to the undefined electron density in the region of the C-terminal CaMD. We assume that high flexibility of CaMD in the absence of calcium is the main reason for unsuccessful structure determination. This was also validated with the determined NMR structure and confirmed with small-angle X-ray scattering measurements (SAXS).

Finally, *in vivo* experiments on human osteosarcoma U2OS cells, performed in association with the group of Prof. Dr. Tea Vallenius from University in Helsinki, revealed that the actin crosslinking activity of α-actinin-1 is regulated by calcium. At low cytosolic calcium concentration actin filaments are associated in tight bundles, while elevated calcium concentration results is destabilization of such bundles.

Key words:

α-actinin-1, calcium, structure, stabilization, crystallization, F-actin filaments