Effects of filamentous actin bundling proteins revealed by EPR spectroscopy

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Abstract Eukaryotes have several highly conserved actin-binding proteins that crosslink filamentous actin (F-actin) into bundles present in cortical and cytoskeletal structures including microvilli, stereocilia and filopodia and are participating in processes as cell-matrix adhesion, cell motility, cell-cell interactions and sarcomere contraction. In our work we studied the changes in filamentous actin following the addition of fascin-1 and alfa-actinin using electron paramagnetic resonance spectroscopy (EPR). Spin labeled maleimide derivative (MSL) was used to observe changes in hyperfine splitting and rotational correlation times due to interaction of actin and the bundling proteins. Evaluation of the EPR spectra suggests that fascin-1 and alfa-actinin-1 induce detectable changes in the rotational freedom of the actin filaments. Interestingly, the two proteins exert their stabilizing effect different way that shows up in the detected hyperfine splitting: alfa-actinin increases it more than fascin-1. In line with the bundling phenomenon, the increase in the hyperfine splitting can be interpreted as a more rigid structure resulting in longer rotational correlation time or can be the consequence of changes in the local microenvironment probed by the MSL caused by the bundling proteins. These results are in agreement with the known different physiological roles of the two proteins. Based on the structural differences of the proteins they can cause different way of actin filament binding that were reflected in different magnitude of change in the EPR signals.

Introduction

There are several actin filament bundling proteins that mediate several processes in cells such as formation of protrusions and cell adhesions on the cell surface, or stress fibers in the cell matrix. Both fascin and alpha-actinin forms stable and rigid actin bundles but it has been shown that despite of similar (crosslinking and bundling) functions alpha-actinin and fascin bound actin filaments display different mechanical properties.

Fascin, as a globular actin filament bundling protein has two actin filament binding sites in one molecule in contrast with alpha-actinin a rod-shaped protein that has only one F-actin binding site but it forms homodimers in a head-to-tail arrangement (Figure 6).

EPR spectroscopy is able to elucidate changes in elasticity on the level of monomers or the filament originating from changes in inter- and intramolecular reactions in F-actin.

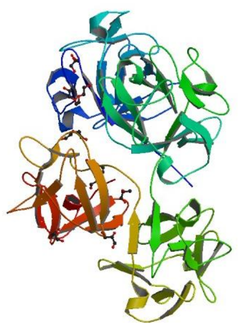

Figure 1:
fascin-1 structure;
pdb code 3P53

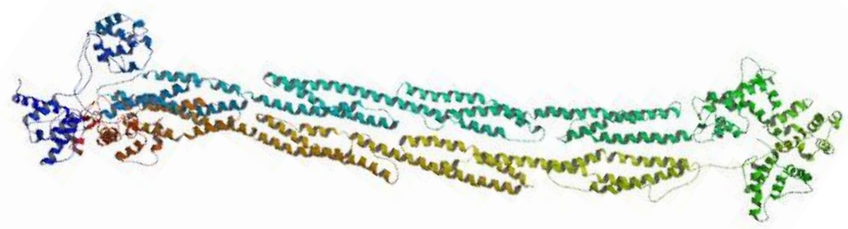

Figure 2:
alpha-actinin-1 structure; pdb code 1SJJ

Results

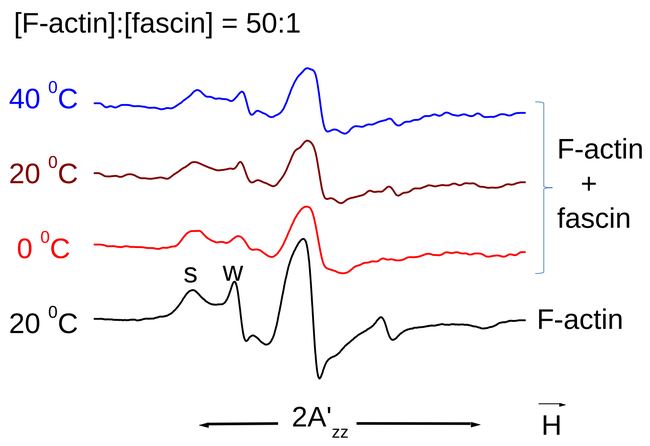

Figure 3: EPR spectra of MSL-F-actin with and without the bundling protein at different temperatures. At increasing temperature the hyperfine splitting constant (2A'zz) decreases, the intensity ratio of the first two peaks (w/s) increases.

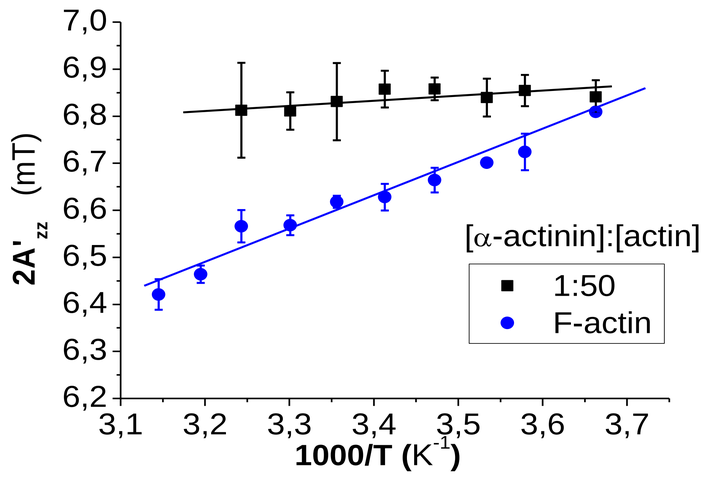

Figure 4: Temperature dependence of 2A'$_{zz}$ plotted against reciprocal absolute temperature at different *[actin]:[α-actinin]* ratios. The received significantly lower slopes in presence of fascin compared to F-actin alone, refers to the increased internal rigidity and likely a higher temperature stability of the actin filaments after binding of the bundling protein alpha-actinin.

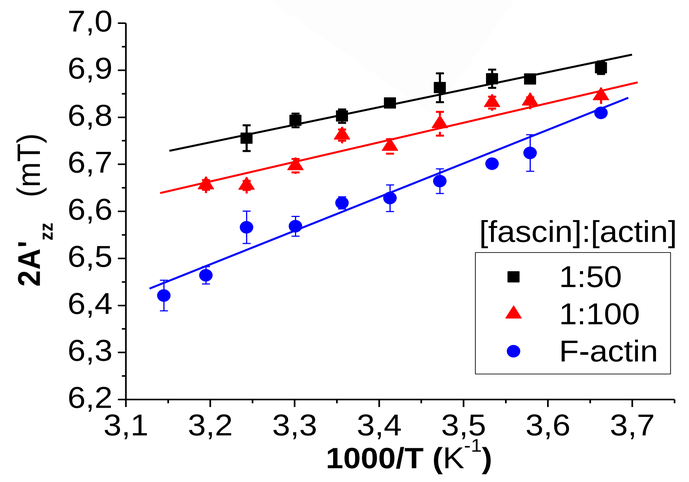

Figure 5: Temperature dependence of 2A'$_{zz}$ plotted against reciprocal absolute temperature. By increasing the *[fascin]* the slope decreases that refers to the increasing internal rigidity of the actin filaments at higher concentration of the bundling protein fascin.

Discussion

- Similarly to F-actin filament alone, we did not observe any break point on the temperature dependent hyperfine splitting constant 2A'$_{zz}$ neither with fascin-actin nor with α-actinin-actin complexes; this indicate the absence of abrupt conformation changes in these macromolecular systems.
- In addition, comparison of the measured 2A'$_{zz}$ values indicates that interaction of both proteins with F-actin results in a more rigid filamental structure at all temperature and protein ratios. This increase in rigidity is more pronounced in case of fascin at lower temperatures, but at higher temperatures the stabilizing effect of the α-actinin becomes stronger. We hypothesize that the different strength of the stabilising effect is due to the different binding and bundling properties of α-actinin and fascin. To get more insight on the role of the stabilizing effects further fluorescence and DSC measurements will be performed in th future.

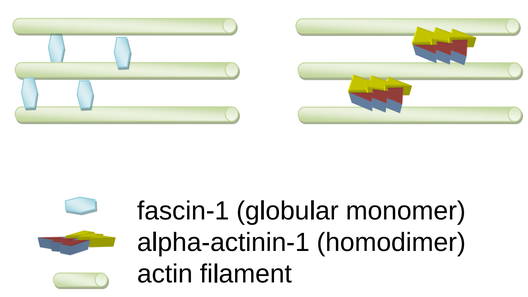

Figure 6: The schematic binding model of fascin and alpha-actinin.

Materials and Methods

Protein purification and modification. Actin was purified from the domestic white rabbit skeletal back and leg muscles

Spin labelling of actin. Actin was spin-labelled with N-(1-oxyl-2,2,6,6,-tetramethyl-4-piperidinyl)-maleimide spin label (MSL) in filamentous form in excess of MSL (a molar ratio of 1:1.2) for 12 hours at 4 °C.

EPR spectroscopy. Conventional EPR spectra of actin were recorded with an ESP 300E X-band spectrometer (Bruker Biospin, Rheinstetten, Germany). First harmonic in-phase, absorption spectra were obtained using 20 mW microwave power and 100 kHz magnetic field modulation with amplitude of 0.15 mT. Actin concentration varied between 30–120 μM; the spectra were recorded at a range of temperature scaling from 0-45 °C increased by steps of 5 °C and kept constant during the measurement with an accuracy of 0.5 °C. The protein samples were placed in two capillary tubes (Mettler ME-18552 melting point tubes), each of them contained 10 μl solution. The sample tubes were positioned parallel in the centre region of the TM 110 cylindrical cavity. A small thermocouple was inserted in one of the capillary tubes, and the temperature was regulated with a diTC2007 type temperature controller.

Computational methods. The EPR spectra was evaluated with the WINEPR program from Bruker and with a computer program developed in our laboratory.

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