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Structural Investigation of the Complex of Actin with Filamin from *Entamoeba histolytica*

Anna Vakhrusheva[1], Samudrala Gourinath[2] and Olga Sokolova[3]

[1]Moscow Lomonosow University, Moscow, Moskva, Russia, [2]School of Life Sciences Jawaharlal Nehru University, Delhi, Delhi, India, [3]Lomonosov Moscow State University, Faculty of Biology, Moscow, Moskva, Russia

Among different infectious diseases, the third largest cause of death has been attributed to *Entamoeba histolytica*. It is a protozoan that causes amoebiasis or amoebic dysentery. It is estimated that around 50 million people are affected by *E .histolytica* worldwide and approximately 50000-100000 deaths are registered every year, especially in developing countries [1]. Phagocytosis is the key process responsible for the pathogenesis of *E. histolytica*, where actin and actin-binding proteins, including filamin, play one of the key roles. Thereby, there is a necessity to understand structural interactions in the complex of actin with filamin from *E. histolytica*.

Filamins are actin cross-linking proteins that hold two actin filaments at large angles and form high-order structures, such as bundles or orthogonal networks. The filamin is composed of an N-terminal ABD (actin-binding domain) followed by 24 immunoglobulin-like domains, the last of which mediates homodimerization [2]. Many structures of the individual domains of filamin are available in the PDB (4B7L, 2WFN, 2WA5, 3FER, 3V8O etc). Recently, the structure of the F-actin and human ABD of filamin was determined by Cryo-EM [3], but still, there is no structure for the filamin from *E. histolytica* with F-actin.

In this work, we elucidated the structure of F-actin with the ABD domain of *E. histolytica'*s filamin. The expression was carried out in *E.coli* cells like BL21 [DE3]. The first step of purification was conducted by affinity chromatography with a Ni-NTA column, and the second step was done by gel filtration chromatography. After that, the purified filamin ABD domain was incubated at 5 μM with 1 μM phalloidin-stabilized F-actin in a low-salt co-sedimentation assay buffer (150 mM KCl) for at least 30 min. For negative staining electron microscopy, 4 μl of this complex was absorbed on the grid and stained with 2% uranyl acetate and visualized in JEOL2100 electron microscope. The diameter of the actin alone was around 7 nm, while for the complex of actin with ABD domain of filamin the diameter of the fibril was around 9 nm, suggesting that the ABD domain of filamin binds to the actin filament. For the determination of the fine structure of the actin-filamin complex, we plan to study the structure with cryo-electron microscopy [4].

References

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