Scale-up Purification and Mild Proteolysis of Myosin Va Globular Tail Domain for Structural Studies

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In spite of the fact the long distance cargoes transport is generally attributed to microtubule-based motors, several studies have been demonstrated the key role of actin-based motors, particularly the class V myosins, including membrane trafficking. The class V myosins are widely distributed since yeast to human and their versatility depends critically on C-terminal globular tail that has marked differences in sequence composition and is responsible for binding to a variety of cargoes and molecular adaptors. Activation and regulation of these molecular motors involve an intrinsically complex multi-conformational states promoted by interactions between regulatory light chains, intramolecular domains, ions and particularly phosphorylation at the globular tail. The aim of this project is shed light on the structural basis of interactions of myosin Va globular tail (GT-Myo5a) and molecular adaptors of cargoes by X-ray structure determination. In this work, we have overexpressed the GST-GT-Myo5a in XL1-Blue strains and optimized a scale-up purification procedure for further structural studies. In-column trypsin digestion has been employed to cleave GST and resulted in two chains of 25 and 20 kDa identified by ESI-MS as C- and N-terminal subdomains, respectively. The subdomains generated by mild proteolysis were analyzed by gel filtration and the polypeptidic chains coeluted as a single peak with corresponding molecular weight of the whole GT-Myo5a. Dynamic light scattering of the digested GT-Myo5a indicated a monodispersive state (<15%) suitable for crystallization trials. The protein was submitted to initial screening by sitting-drop vapor-diffusion technique testing robotically 650 crystallization conditions. Preliminary results indicate the presence of several micro-crystals that will be used for further grid screen optimization.