Intramolecular interaction of adducin C-terminal to regulate its actin-binding function

E. Bódis[1], R. Judd[2,3], M. R. Bubb[2,3,*]
[1]University of Pécs, Medical School, Department of Biophysics, emoke.bodis@aok.pte.hu
[2]University of Florida, Department of Medicine in the College of Medicine, Rheumatology and Clinical Immunology
[3]Malcom Randall VA Medical Center, Gainesville, Florida

Keywords: *adducin, autoinhibition, spectrin, fluorescence spectroscopy*

Abstract

Adducin is a 110 kDa actin-binding protein which known functions are the capping of the fast growing end of the actin filaments and its ability to recruit spectrin to the actin filaments in nerve cells and red blood cells. Adducin is composed of a C-terminal unfolded domain, an alpha-helical neck domain and an N-terminal tertiary structured head domain which function is still debating. The recognized functions of adducin are related to the very end of the C-terminus. The last 25 amino acids form a phosphorylation side domain (PSD), which is mainly consisted of basic amino acids. PSD of adducin is homologue of the PSD of MARCKS, which is established that able to form an intramolecular interaction that regulates MARCKS's required function. We hypothesize that PSD of adducin has a similarly regulated mechanism than MARCKS has.

Using methods of fluorescence spectroscopy we found that PSD alone (synthetized peptide corresponding to the PSD in adducin) is more active in actin nucleation and the inhibition of actin filament depolymerization than PSD embedded in the C-terminal domain. We confirmed that PSD has an intramolecular binding site within the C-terminal domain. We could show that the 32 amino acids long region of the C-terminus is mainly built of acidic amino acids and can attach by salt bridges to PSD. This auto-regulation domain (AID) reduces the active function of PSD by binding. Adding spectrin (the main component of membrane skeletal system of the red blood cells and nerve cells) to the PSD-AID complex it can disrupt their binding. Our experiments revealed that the strong binding of spectrin and AID liberates PSD from the blocking effect of AID to fulfil its actin nucleating and capping function.