Abstract

Post-translational modifications through ubiquitination play a crucial role in the regulation of membrane proteins. Nedd4-2, a human HECT E3 ubiquitin ligase is the last component of the ubiquitination cascade that transfers the ubiquitin molecules and triggers the endocytosis of its downstream target molecules. Dysregulation of Nedd4-2 can cause various disorders, including epilepsy, respiratory distress, and Liddle syndrome. Despite the involvement of different adaptor proteins in the regulation of Nedd4-2, our focus in this research was on the conserved 14-3-3 proteins, known negative regulators of Nedd4-2. In this study, we performed biophysical characterization of Nedd4-2$^{190–581}$ and Nedd4-2$^{186–975}$ constructs while in complex with 14-3-3 to get further insight into the dynamics of this interaction. Our results from time-resolved fluorescence spectroscopy revealed that 14-3-3 binding impacts the emission properties and mobility of specific WW domains (WW3 and WW4) of Nedd4-2, while sparing others (WW1). Intriguingly, the catalytic HECT domain undergoes conformational changes and increased solvent exposure upon complex formation. We propose that steric hindrance of WW3 and WW4 domains, combined with conformational alterations in the catalytic domain, may underlie the regulatory mechanism mediated by 14-3-3 binding. Chemical cross-linking coupled with mass spectrometry and limited proteolysis experiments further elucidate extensive structural changes in Nedd4-2 domains in the presence of 14-3-3 proteins, highlighting the protective role of 14-3-3 against proteolytic degradation. Overall, our comprehensive findings shed light on the intricate molecular mechanisms governing the 14-3-3 binding-mediated regulation of Nedd4-2, offering valuable insights into the better understanding of ubiquitin-mediated regulation of membrane protein functions and its possible role in various pathological conditions.