Abstract EN

The human ubiquitin ligase Nedd4-2 (NEDD4L) ubiquitinates a wide range of membrane proteins and receptors, playing a key role in maintaining homeostasis. This enzyme is regulated by phosphorylation and subsequent interaction with 14-3-3 proteins, which primarily affects its ability to interact with various substrates. However, very little is known about the molecular basis of this protein-protein interaction. In this work, we focused on biophysical characterization of the role of individual phosphorylation sites and also on mapping the structural changes in the Nedd4-2 protein induced by 14-3-3 protein binding. Our experiments using analytical ultracentrifugation methods revealed that two phosphorylation sites Ser^{342} and Ser^{448} are primarily required for stable binding of Nedd4-2 to 14-3-3 proteins. The crystal structure of the 14-3-3ηΔC:Nedd4-$2^{335-455}$T367A complex than revealed the simultaneous binding of both phosphorylated residues to the binding groove of 14-3-3 protein. Subsequent modeling based on small-angle X-ray scattering and chemical cross-linking data combined with mass spectrometry indicated extensive structural changes in the individual domains of the Nedd4-2 protein. Binding of 14-3-3η protein blocks the WW3 domain of Nedd4-2 in the central channel of 14-3-3 protein, while altering the relative positions of the WW2 and WW4 domains. The WW domains of Nedd4-2 are responsible for downstream substrate recognition, and changes in the accessibility of individual WW domains may thus elucidate how 14-3-3 protein modulates the ubiquitination of some Nedd4-2 substrates. The findings provide the first structural insight into the molecular basis of the mechanism of Nedd4-2 protein regulation by 14-3-3 proteins.