Characterization of the Dependence of Src:ND2 Binding on Phosphorylation and Intramolecular Src Interactions

Abstract

NMDA receptor is implicated in many disease processes in the central nervous system, including neurodegenerative disorders, epilepsy, chronic pain, and stroke. The role of the non-receptor tyrosine kinase Src in up-regulating NMDAR activity via phosphorylation of the carboxyl-terminal domain of NMDAR has been extensively studied. The interaction between the intrinsically disordered regions of Src and an anchoring protein, ND2, has been discovered to be essential in this up-regulation. However, the molecular mechanism underlying this docking remains to be elucidated. Herein we used NMR, fluorescence, and bio-layer interferometry to characterize the binding between the ND2 cytoplasmic loop and various Src construct lengths with different sites phosphorylated. We have demonstrated that the interaction between Src and ND2 loop is phosphorylation regulated, resulting in an enhancement from no detectable binding to sub-micromolar tight binding, especially with phosphorylation at Thr37/Ser75. Moreover, we have discovered that the presence of SH3 domain increases binding affinity significantly from a sub-millimolar to sub-micromolar level. These results indicate how electrostatic interactions and intramolecular Src interactions between SH3 and the disordered regions of Src influence the Src:ND2 interaction. Our findings expand knowledge of the function and regulatory possibilities of disordered proteins in general, and will be valuable for the development of therapeutic agents that target the Src:ND2 complex to treat the patients suffering from chronic pain.