Abstract

Gephyrin is the major scaffolding protein which develops a lattice underneath the postsynaptic membrane of inhibitory synapses. It clusters and immobilizes Glycine receptors (GlyRs) and γ-aminobutyric acid receptors ($GABA_ARs$) in the postsynaptic membrane by interaction with the cytoskeleton. At the same time gephyrin also interacts with certain other proteins which play a regulatory role in synaptic clustering. Amongst others gephyrin binds to the dynein light chain 1 and 2 (DLC1 and 2) of the LC8 family which are components of the cytoplasmic dynein motor complex and the myosin V motor.

The crystal structure of gephyrin G and E domain reveal a trimeric and dimeric composition. The structure of the central C domain remains unknown. For a better understanding how the molecular scaffold of gephyrin is established at the postsynaptic membrane the knowledge of the structural formation of the C domain is necessary. DLC1 and 2 bind gephyrin in the N-terminal region of the C domain. After expression and purification of the proteins the stability of gepyhrin was analyzed in the absence and presence of DLC 1 and 2 by partial proteolysis and differential scanning calorimetry (DSC). The results showed that the C domain is rapidly degraded that might result from a surface exposed location in the overall structure and an unstructured formation. Binding of DLC1 and 2 lead to an increased stability. Based on these results truncated gephyrin variants were cloned and expressed which were used for crystallization but unfortunately no crystals were obtained. Detailed binding parameters from isothermal titration calorimetry and analytical gelfiltration showed a 3 : 2 stoichiometry of the gephyrin / DLC1 complex. This ratio shifted to 3 : 1 in the complex of the truncated gephyrin variants and DLC1 as well as for the isolated C domain with DLC1.

The DLC mediated transport is based on a simultaneous binding of DLC1 to cargo molecules and the dynein motor complex. Cosedimentation experiments, analytical gelfiltration and colocalization in non neuronal cells disprove the formation of a ternary complex established by gephyrin, DLC1 and dynein intermediate chain 1 and 2 (DIC1 and 2) which makes a DLC mediated transport of gephyrin unlikely.

To investigate the function of gephyrin and DLC1 in more detail variants of both proteins were established which are not able to bind each other. Colocalization in Cos-7 cells showed a reduced binding of gephyrin and DLC1. The analysis of these variants in hippocampal neurons showed a reduced localization of gephyrin cluster at the synapse which might indicate a regulatory function of the interaction of gephyrin and DLC.