Abstract

Gephyrin is the key scaffolding protein at inhibitory synapses in the central nervous system where it anchors glycine (GlyR) and a subset of γ-aminobutyric acid type A ($GABA_A$) receptors in the postsynaptic membrane. The protein is composed of three domains with crystal structures of N-terminal G- and C-terminal E-domain as trimers and dimers, respectively. Despite the lack of structural information for gephyrin's central C domain and the full-length protein, a hexagonal lattice of gephyrin has been proposed underneath the post-synaptic membrane which lacks experimental approval, as *E. coli* expressed gephyrin forms exclusively trimers in solution. Additionally the gephyrin gene undergoes tissue and species-specific alternative splicing resulting in various gephyrin isoforms, with only little knowledge about their functional properties.

In this work an eukaryotic expression system for three full-length gephyrin splice variants (Geph, Geph-C3, Geph-C4) has been established in Sf9 insect cells and folding, oligomerization, receptor binding and posttranslational modifications has been investigated. In contrast to *Escherichia coli* expressed gephyrin, Sf9 gephyrins form hexamers as basic oligomers. In addition, the formation of higher oligomers (approx. 900 kDa) was also observed for Geph and Geph-C4. Partial proteolysis and differential scanning calorimetry revealed a compact folding of the Gephyrin G and C domain in one complex, whereas a much lower stability for the E domain was found. Following GlyR β-loop binding, the stability of the E domain increased in Geph and Geph-C4 significantly. In contrast, the E-Domain in Geph-C3 is less stable and binds the GlyR β-loop with two orders of magnitude lower affinity as confirmed by isothermal titration calorimetry. Based on these results two models for gephyrin oligomerization with trimer interaction either via the C and E domains has been proposed.

In the second part of this work posttranslational modifications and their functional impact on gephyrin clustering have been investigated. Peptide mass fingerprinting of Sf9-derived gephyrins revealed 18 novel phosphorylation sites, of which all, except one are located within the C domain. Following a bioinformatic analysis for potential kinase consensus motifs in gephyrin, *in vitro* phosphorylation and subcellular localization studies in hippocampal neurons in absence and presence of kinase inhibitors, have been conducted. Besides GSK3, a recently idenfied gephyrin-kinase, casein kinase II and mitogen-activated Proteinkinase Erk were found to influence gephyrin clustering. Mutagenesis of different gephyrin phosphorylation-sites identified a novel phosphorylation site (T324) in the gephyrin E-domain, which impacts gephyrin oligomerization and clustering in non-neuronal cells as well as hippocampal neurons. While the phospho-mimetic variant Geph-C4 T324D showed an altered oligomerization behavior following expression in Sf9 insect cells, the phospho-deficient variant Geph-C4 T324A showed spike-like structures that were resolved in the presence of two gephyrin ligands that bind within or in close proximity to a motif hosting T324. In aggregate, T324 seems to be crucial in gephyrin oligomerization and clustering and might point to the still unknown site of gephyrin multimerization during receptor clustering.