ZINC IN THE BRAIN: UNRAVELLING THE ROLE OF ZINC-METALLOTHIONEIN-3

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Neuronal growth inhibitory factor, metallothionein-3 (Zn_7MT-3), represents a major component of the intracellular zinc pool in zinc-enriched neurons. The protein impairs the survival and neurite formation of cultured neurons and is downregulated in Alzheimer's disease brains. With the aim of gaining insights into the mode of action of Zn_7MT-3 at the molecular level, we have conducted structural and biological studies on recombinant M^{II}_7MT-3 and its mutants. By using EXAFS, MCD and ^{113}Cd NMR of M^{II}_7MT-3 the existence of highly dynamic $M^{II}_4(CysS)_{11}$- and $M_3(CysS)_9$-clusters localized in two mutually interacting α- and β-domains, respectively, was demonstrated. Mutational analysis established that the T(5)-C-P-C-P(9) motif in the N-terminal β-domain is critical for the extracellular inhibitory activity of MT-3 and unprecedented dynamics of its $M^{II}_3(CysS)_9$-cluster.

In zinc-enriched neurons a large amount of chelatable Zn^{II} is sequestered in presynaptic vesicles and, upon stimulation, released into the synaptic cleft through the process of exocytosis. The exo-endocytotic cycle of synaptic vesicles in neurons is strictly linked to the small GTPase Rab3A. The role of co-localized Zn_7MT-3 in the intracellular trafficking of Zn^{II} is not understood. By using affinity precipitation, surface plasmon resonance (SPR), and fluorescence techniques we showed that Zn_7MT-3 binds reversibly to Rab3A·GDP (K_d = 2.6 μM), but not to Rab3A·GTP. The binding of Zn_7MT-3 to Rab3A·GDP is specific as no binding was observed with the predominantly unstructured metal-free form of MT-3, suggesting that the well-defined metal dictated MT-3 fold is essential for binding. To map for the interaction site on the Rab3A structure the binding of Zn_7MT-3 to the C-terminally truncated Rab3A(2-186) mutant was examined. The substantially lower binding affinity mapped the interaction surface to the effector binding site of Rab3A. This location is further supported by kinetics of the GDP exchange, which was found unaffected by Zn_7MT-3 binding to Rab3A·GDP. The interaction of Zn_7MT-3 with Rab3A indicates that MT-3 is not merely a cellular Zn^{II} buffer, but actively participates in synaptic vesicle trafficking upstream of vesicle fusion. The implications of these studies for the biological function of Zn_7MT-3 will be presented.