Structural insights into the calmodulin-Munc13 interaction obtained by cross-linking and mass spectrometry.


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Munc13 proteins are essential regulators of synaptic vesicle priming and play a key role in adaptive synaptic plasticity phenomena. We recently identified and characterized the Ca(2+)-dependent interaction of Munc13 and calmodulin (CaM) as the molecular mechanism linking changes in residual Ca(2+) concentrations to presynaptic vesicle priming and short-term plasticity. Here, we used peptidic photoprobes covering the established CaM-binding motif of Munc13 for photoaffinity labeling (PAL) of CaM, followed by structural characterization of the covalent photoadducts. Our innovative analytical workflow based on isotopically labeled CaM and mass spectrometry revealed that, in the bound state, the hydrophobic anchor residue of the CaM-binding motif in Munc13s contacts two distinct methionine residues in the C-terminal domain of CaM. To address the orientation of the peptide during binding, we obtained additional distance constraints from the mass spectrometric analysis of chemically cross-linked CaM-Munc13 peptide adducts. The constraints from both complementary cross-linking approaches were integrated into low-resolution three-dimensional structure models of the CaM-Munc13 peptide complexes. Our experimental data are best compatible with the structure of the complex formed by CaM and a CaM-binding peptide derived from neuronal NO synthase and show that Munc13-1 and ubMunc13-2 bind to CaM in an antiparallel orientation through a 1-5-8 motif. The structural information about the CaM-Munc13 peptide complexes will facilitate the design of Munc13 variants with altered CaM affinity and thereby advance the detailed functional analysis of the role of Munc13 proteins in synaptic transmission and plasticity.