Study of binding partners and modulation of calmodulin functional properties and its fusion protein variants

Abstract

The thesis studies calmodulin (CaM)-dependent modulation of the TRPM ion channel subfamily involved in the pathogenesis of cardiovascular, neurodegenerative diseases or cancer. In total, 5 new CaM-binding epitopes were characterized in detail in TRPM4, TRPM5, TRPM6, and TRPM7. The results of molecular modeling and docking showed good accessibility of the binding epitopes for an interaction with CaM. The presence of basic residues, typical for non-covalent CaM interactions with TRP, was required for the formation of complexes with CaM. As regards TRPM5, TRPM6, and TRPM7, this is the first study that indicates their potential modulation by CaM. Furthermore, CaM was studied in terms of possible optimization of the properties of the molecule in fusion protein constructs. The innovative fusion molecule CaM/AMBN-Ct was obtained by fusing CaM and an intrinsically disordered C-terminal domain of ameloblastin (AMBN-Ct). As shown by circular dichroism (CD) spectroscopy and binding studies, the required structural and functional properties of CaM are preserved in CaM/AMBN-Ct. The results of sedimentation analyses and CD spectroscopy indicated communication between the fusion partners. Mutual contacts between CaM and AMBN-Ct in the fusion molecule resulted in a significant increase in thermal stability of CaM. The CaM/AMBN-Ct fusion construct thus may be used for the design of more stable molecules based on unique CaM properties, for biomedical and biotechnological applications.

Keywords

Ameloblastin, calmodulin, fusion proteins, intrinsically disordered proteins, protein engineering, TRPM ion channels