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Florida International University

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Abstract

Allosteric mechanism on DREAM dimerization and impact on its interactions with divalent metal Zn^{2+}.

by

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DREAM is a member of the NCS family which is mainly present in the hippocampus. It has been involved in memory,learning , pain sensitivity and gene expression and it has been linked to Alzheimer's and Parkinson's disease. DREAM interactions are mediated by Ca^{2+} association to the protein which allosterically modify its affinity to other intracellular partners such as DNA and $K_{v4.3}$ channels , and result in changes in its oligomerization state. The objective of our study was to characterize the mechanism of Ca^{2+} binding, its role in allosteric control of effector proteins association and the impact of Zn^{2+} on functional properties of this protein. A kinetic study of the binding of Ca^{2+} to DREAM has shown that dimerization is a two-step process, the first step (τ_1= 8±0.1ms) is associated with the binding of Ca^{2+} to the monomer, while the second step (τ_2 = 3.6 ±0.4 s) corresponds to a conformation relaxation that leads to the formation of the dimer. Inverse hyperbolic dependance of the rate constant for Ca^{2+} binding on the metal concentration demonstrated that Ca^{2+} recognition occurs through a conformational selection mechanism providing crucial information on the binding of Ca^{2+} to DREAM. MD analyses were performed to determine Trp169 role in a network of hydrophobic residues in DREAM that participates in the transmission of the interdomain allosteric signal. The mutation of Trp to Ala lead to a loss of structural rearrangement. For DREAMW169A the structure stabilizing salt bridge K87-E165 was not present , the EF hands in the mutant did not undergo the reorientation observed in DREAMWT and the dimer divided into two Ca^{2+}bound monomers, suggesting the Trp169 is involved in interdomain communication, and it is crucial for the protein proper functioning. Zn^{2+} is involved in several vital physiological processes and interacts with various proteins , including other NCS . DREAM also interacts with divalent metals with relatively high affinity . The presence of Zn^{2+} produces changes in DREAM's tertiary structure , and it is able to bind to DREAM in the presence(K_d=10.76± 1.46 and µM) and absence of Ca^{2+} (K_d=6.9 ± 0.6 µM). These findings suggest that DREAM as well as other NCS may be involved in Zn^{2+} neurotoxicity. The results from this study provide a higher insight into DREAM structure and function and are very likely to be applied to other NCS members.

Date: June 29th , 2022 **Department:** Chemistry and Biochemistry

Time: 2:00 pm **Major Professor:** Dr. Jaroslava Miksovska

Place: CP-320