Calcium Signaling Proteins

3518-Pos

Characterization of Conformational Transitions in Dream Protein using Fluorescence Spectroscopy

Gangadhar Dhulipala, Jaroslava Miksovska.
Florida International University, Miami, FL, USA.

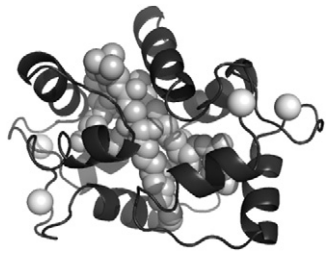
DREAM (Down Stream Regulatory Antagonist Modulator) belongs to an important class of calcium binding proteins that are involved in transducing the calcium signal into a biological response in neuronal tissue. DREAM is a unique calcium binding protein that directly binds to DNA and regulates the transcription of prodynorphin and c-*fos* genes in a calcium dependent fashion. Here we report the conformational transitions in DREAM upon calcium binding by monitoring fluorescence properties of tryptophane residue which is located in proximity to the high affinity calcium binding EF-hand 3. Ca^{2+} association to the protein leads to the hypsochromic shift in the Trp emission spectrum ($_{max}$ = 338nm) compared to the apo form ($_{max}$ = 343 nm) indicating that the calcium induced conformational transition strongly alters fluorescent probe environment. The changes in the Trp emission spectra are strongly influenced by DREAM concentration confirming that alteration of oligomerization state of DREAM can be readily monitored using fluorescence data. The ligand induced changes in the tryptophan environment were further confirmed by the fluorescence quenching studies using iodide and acrydine quenchers. Trp properties were also analyzed using fluorescence lifetime measurements in frequency domain. In both forms, apo- and Ca2+ bond, the tryptophan lifetime can be described using two exponential decay model , with $_1$ = 1.5 ns and $_1$=3.5 ns. The Ca^{2+} association to protein does not significantly alter tryptophan lifetime but increase the fraction of protein with the lifetime of 1.5 ns.

3519-Pos

New Aspects of Lipid-Protein Interactions Revealed by Calmodulin Binding to the Lipid Mediator Sphingosylphosphorylcholine

Erika Kovacs[1], Veronika Harmat[2], Judit Toth[1], Beata G. Vertessy[1], Karoly Modos[3], Jozsef Kardos[2], Karoly Liliom[1].
[1]Institute of Enzymology, Budapest, Hungary, [2]Eotvos Lorand University, Budapest, Hungary, [3]Semmelweis University, Budapest, Hungary.

Lipid-protein interactions drive core cellular signaling events and regulate the function of most membrane proteins. Despite their significance, molecular details of these interactions are scarcely known. Here we provide mechanistic insight into the binding of the lipid mediator sphingosylphosphorylcholine to calmodulin, the most central and ubiquitous regulator protein in calcium signaling, and present a crystal structure of their complex. Thermodynamic and kinetic analysis of their interaction revealed a peculiar stoichiometry-dependent binding process. At low protein-to-lipid ratios, calmodulin binds with high affinity to lipid micelles. At high protein-to-lipid ratios, however, the resulting complex adopts a compact globular conformation with calmodulin embracing a few lipid molecules, as can be seen in our crystal structure. Intriguingly, the sphingolipid occupies the binding pocket for the amphipatic alpha-helices of calmodulin's target proteins. This finding explains the competitive inhibition of calmodulin function by the signaling lipid, and proposes an utterly novel type of endogenous regulation for the calcium sensor protein. The binding model presented here might be widely applicable to other lipid-protein interactions as well.



3520-Pos

Substitutions for a Semi-Conserved IQ Motif Glycine Differentially Affect the Stabilities of Different Ca^{2+}-Bound States of a Calmodulin-IQ Domain Complex

Anthony Persechini, DJ Black.
University of Missouri-Kansas City, Kansas City, MO, USA.

We have determined the effects of substituting Ala, Arg or Met for a semi-conserved Gly residue on the stabilities of different Ca^{2+}-bound states of a reference calmodulin-IQ domain complex. The substitutions are all destabilizing in the absence of Ca^{2+}, with free energy increases of ~2 kJ/mol for the Ala and Arg substituted complexes, and ~6 kJ/mol for the Met substituted complex. When Ca^{2+} is bound only to the C-ter pair of Ca^{2+}-binding sites in calmodulin the free energy of the Ala substituted complex is essentially unaffected, and the free energies of the Arg and Met substituted complexes decrease by ~4 and ~6 kJ/mol. In contrast, the free energy of the reference complex increases by ~4 kJ/mol. When the N-ter Ca^{2+}-binding sites in the intermediate C-ter Ca^{2+}-bound reference and substituted complexes are occupied their free energies all decrease by ~4 kJ/mol. This suggests that the N-ter calmodulin lobe undergoes a similar conformational change in all the complexes, which therefore does not involve direct interactions with the semi-conserved position. When Ca^{2+} is bound only to the N-ter pair of Ca^{2+}-binding sites the free energy of the Arg substituted complex is unaffected, but the free energies of the Ala and Met substituted complexes increase by ~13 and ~9 kJ/mol, and the free energy of reference complex increases by ~7 kJ/mol. This is consistent with a destabilizing interaction between the Ca^{2+}-bound N-ter calmodulin lobe and the calmodulin-binding sequence that requires a non-polar residue at the semi-conserved position. Our results are overall consistent with a model in which the N-ter calmodulin lobe has two different binding sites on the reference CaM-binding sequence, one of which contains the semi-conserved Gly position.

3521-Pos

Conformational Changes of CaMKII: A Model of Activation

Laurel Hoffman, Kristian Kaufmann, Roger Colbran, Jens Meiler, Hassane Mchaourab.
Vanderbilt University, Nashville, TN, USA.

The ubiquitously expressed calcium/calmodulin dependent protein kinase II (CaMKII) functions as a transducer of calcium (Ca^{2+}) signaling by responding to the amplitude, duration, and frequency of Ca^{2+} transients. Autophosphorylation at Thr286 following binding of calcium-calmodulin (Ca^{2+}/CaM) leads to a Ca^{2+}-independent activity referred to as a conformational memory of prior activation. While CaM binding, autophosphorylation, and catalytic-regulatory domain autoinhibition have been linked to CaMKII function, the underlying structural and dynamic framework of activation and conformational memory is poorly understood. Here we utilize site-directed spin labeling and electron paramagnetic resonance (SDSL-EPR) to explore the conformational changes associated with CaMKII activation and conformational memory. The structure of the regulatory domain was investigated via spin label mobility under several conditions representing various intermediates of activation. Inter-domain movements were also examined through distance measurements between regulatory and catalytic domains. We found that CaMKII activation is associated with regulatory-catalytic domain disengagement, causing a disruption of autoinhibition and producing significant conformational changes which are propagated throughout the regulatory domain. Here we detail a mechanistic description of activation and are currently using EPR data to computationally model conformational changes associated with CaMKII activation.

3522-Pos

Anomalous Calcium Dependent Binding of Calmodulin to KCNQ2 Potassium Channels

Alessandro Alaimo[1], Pilar Areso[2], **Alvaro Villarroel**[1].
[1]Unidad de Biofísica CSIC-UPV/EHU, Leioa, Spain, [2]Universidad del País Vasco, Leioa, Spain.

Often, the free concentration of the ubiquitous Ca^{2+} sensor calmodulin (CaM) is limiting, probably below 50 nM in excitable cells, because most of it is bound to targets inside the cell. However, it is generally assumed that the binding characteristics do not depend on CaM concentration, and it has been generally studied at much larger concentrations. We show here that this assumption does not hold true. We have found that Ca^{2+} favors or reduces the association between KCNQ2 potassium channels and CaM depending on the free CaM concentration, shifting abruptly between negative and positive cooperativity when the concentration of CaM is below or above ~26 nM, respectively. With CaM concentrations below 26 nM there was a ~four-fold decrease in the binding affinity in the presence of Ca^{2+}, shifting the Kd from ~4.7 to ~22 nM. In contrast, with CaM concentrations above 26 nM there was a ~two-fold increase in the binding affinity in the presence of Ca^{2+}, shifting the Kd from ~54 nM to ~22 nM. In addition, when 12.5 nM CaM was used in the assay, the EC_{50} for Ca^{2+} binding changed from ~302 nM to ~608 nM or ~645 nM in the presence of molar excess of the KCNQ2 or KCNQ3 CaM binding sites, respectively. In contrast, using 100 nM CaM in the assay, the affinity for Ca^{2+} binding increased slightly in the presence of molar excess of the KCNQ2 binding protein, lowering the EC_{50} to ~287 nM. The anomalous CaM-dependent behavior of CaM adds complexity to the integration of calcium signaling inside the cell.